FIG. 1

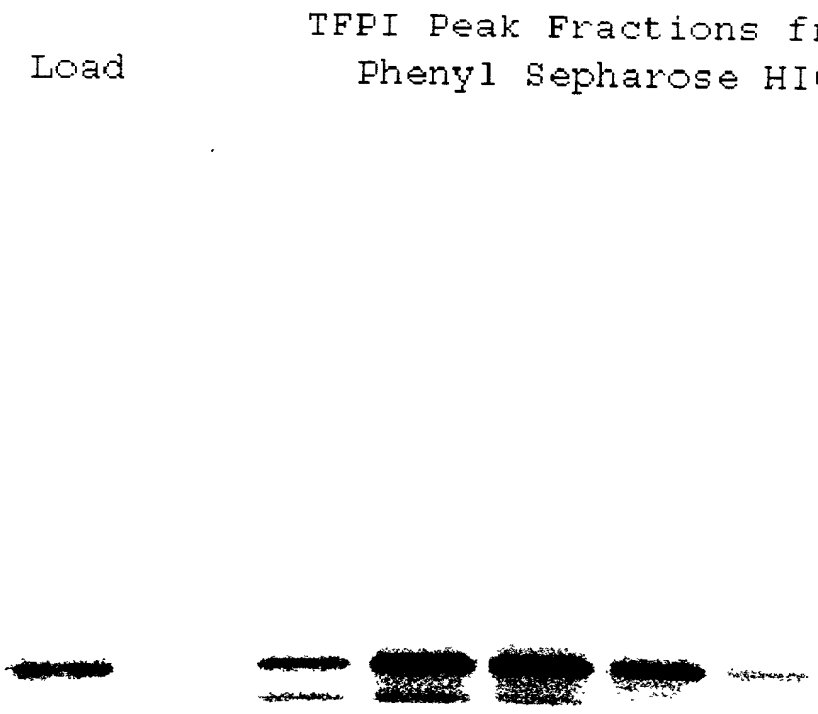


FIG. 2

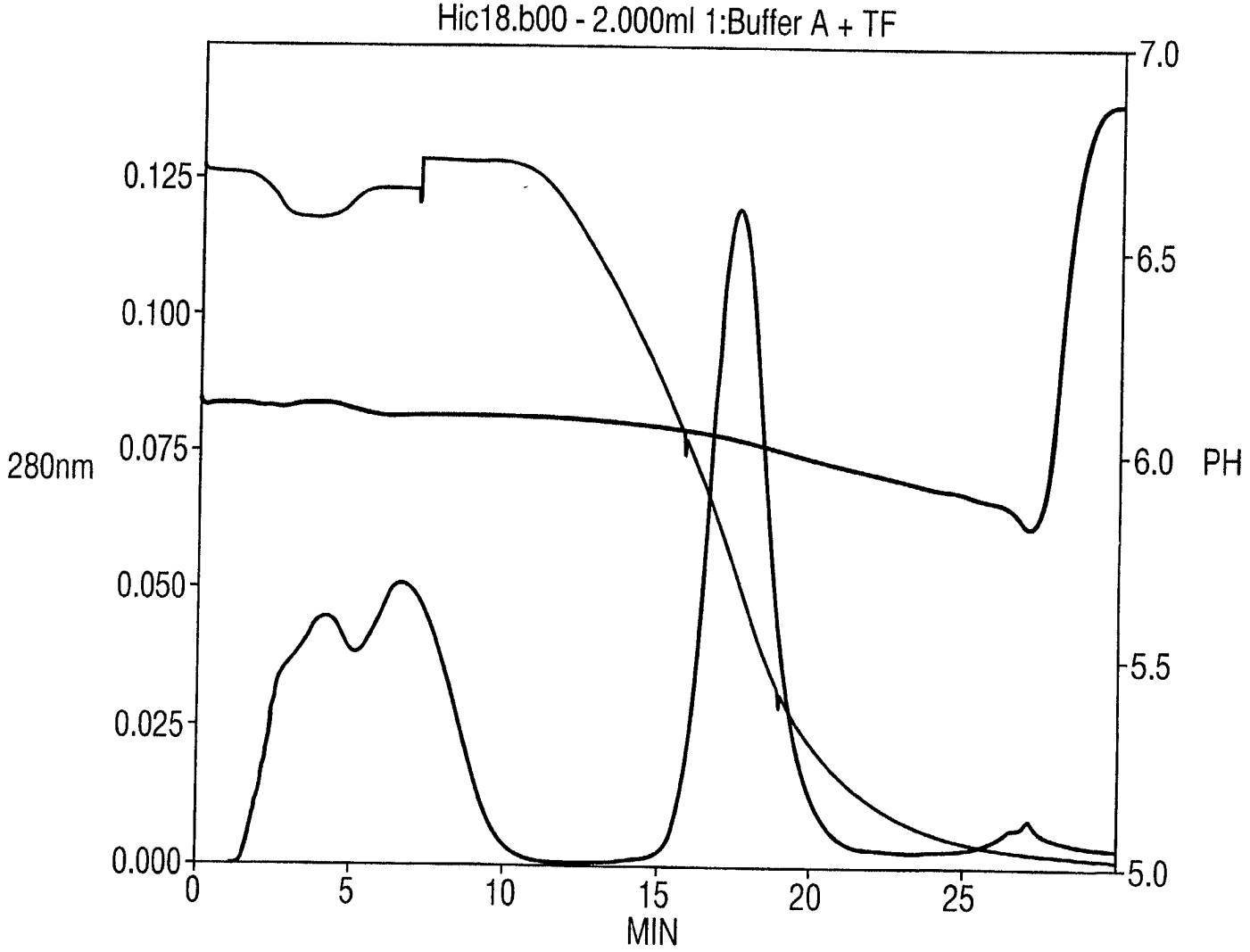

Hic18.b00 - 2.000ml 1:Buffer A + TF
280nm
0.125
0.100
0.075
0.050
0.025
0.000
0
5
10
15
20
25
MIN
7.0
6.5
6.0
5.5
5.0
PH

FIG. 3

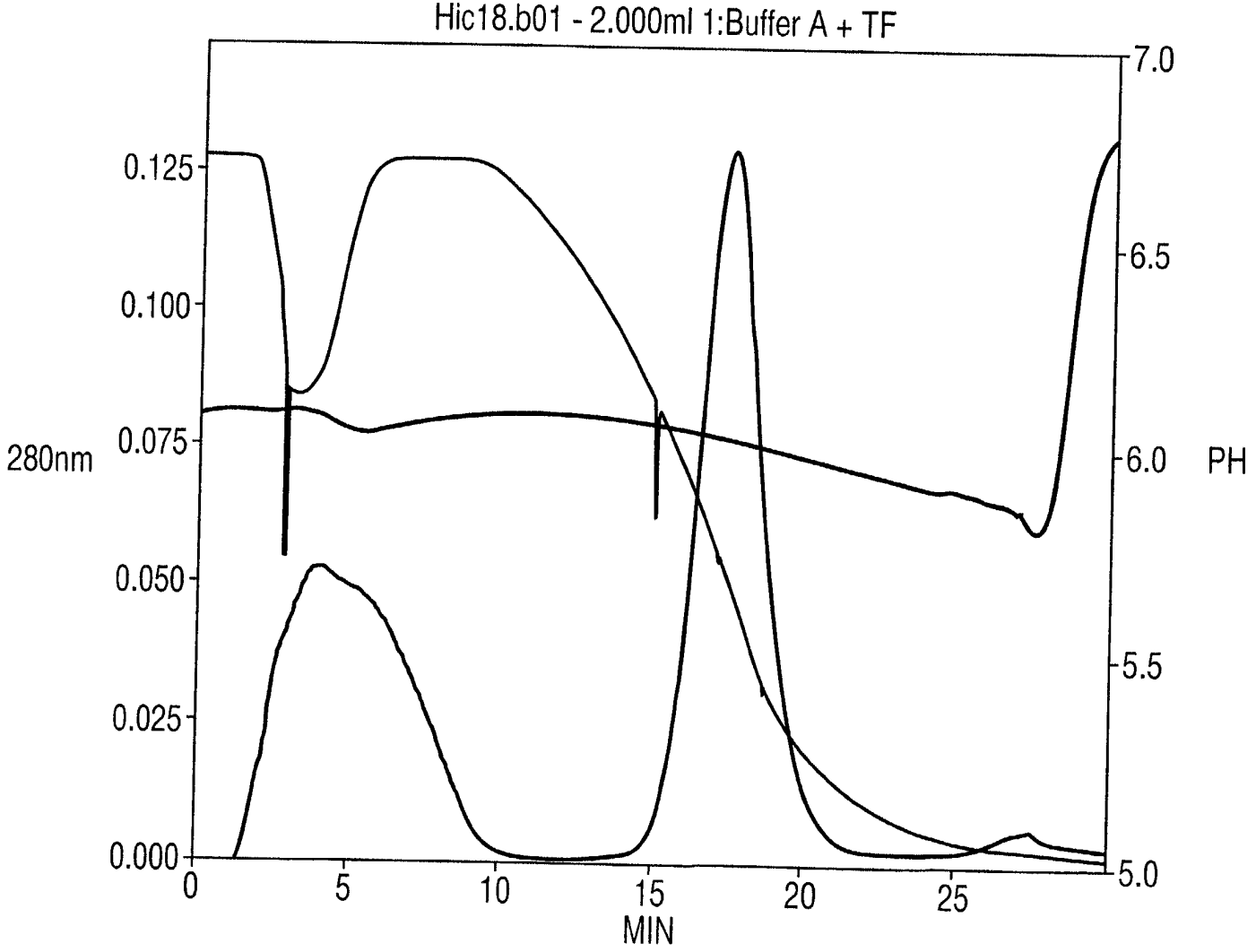

Hic18.b01 - 2.000ml 1:Buffer A + TF
280nm
0.125
0.100
0.075
0.050
0.025
0.000
7.0
6.5
6.0
5.5
5.0
PH
0
5
10
15
20
25
MIN

FIG. 4

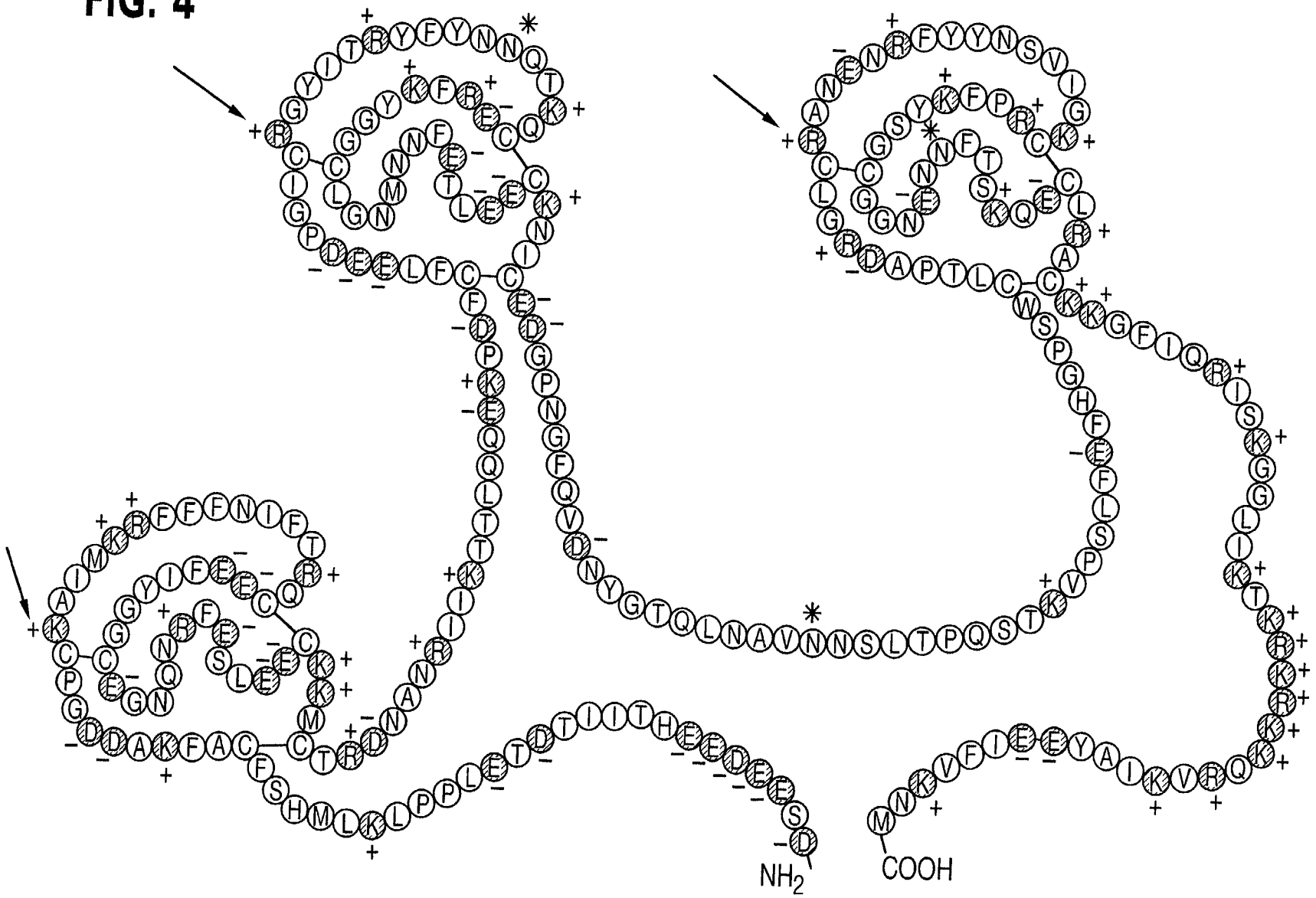


FIG. 5

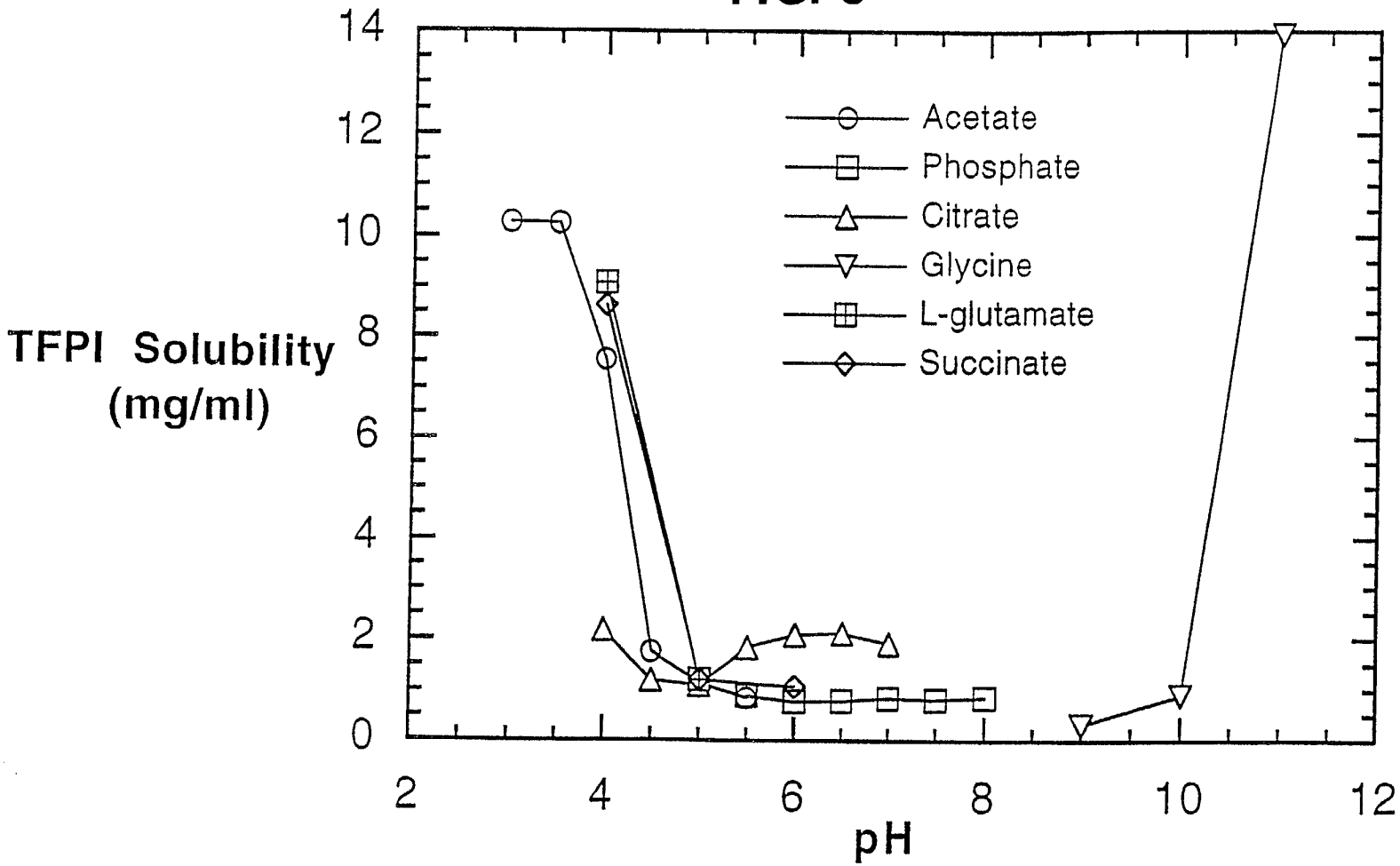


FIG. 6

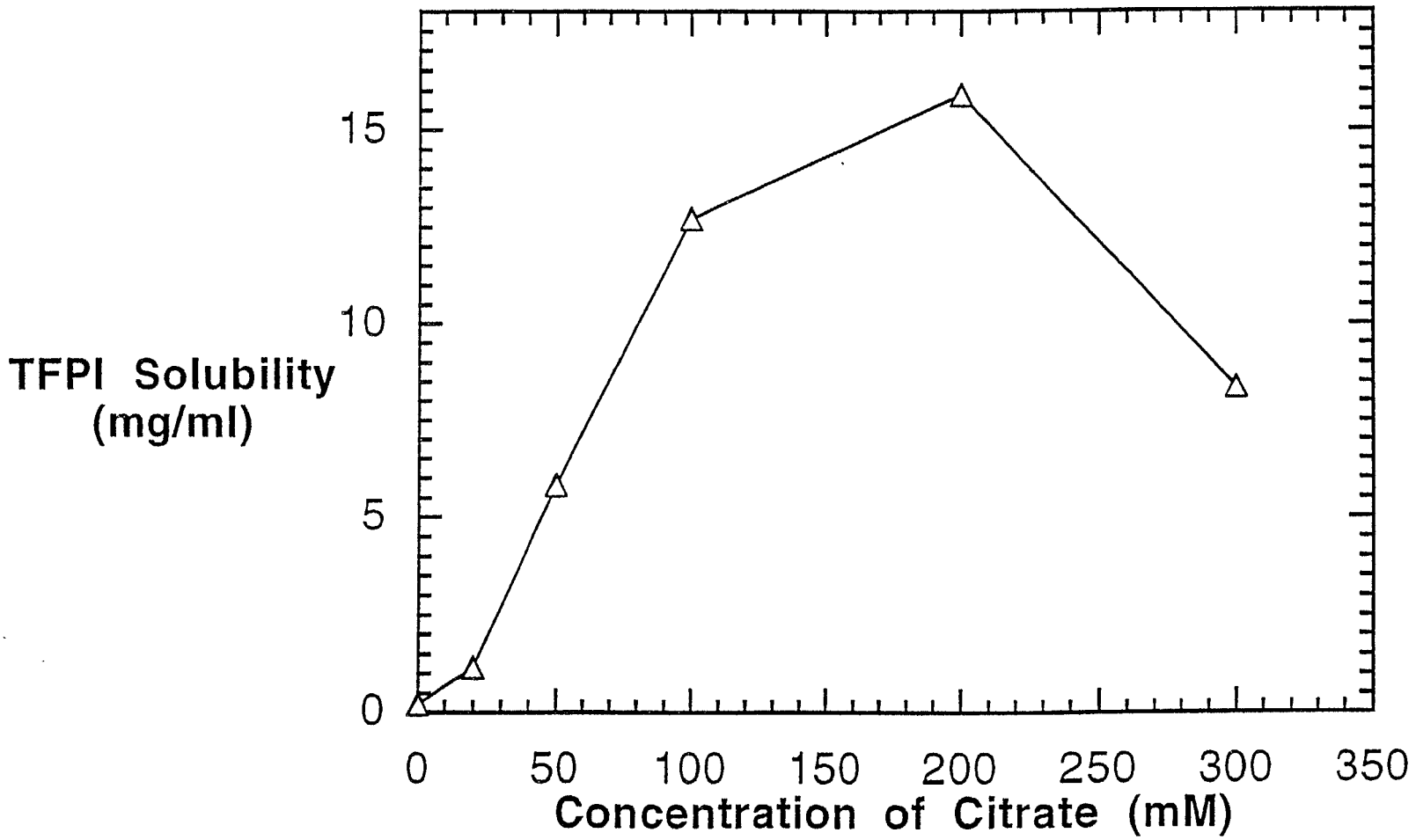

TFPI Solubility
(mg/ml)
15
10
5
0
0
50
100
150
200
250
300
350
Concentration of Citrate (mM)

FIG. 7

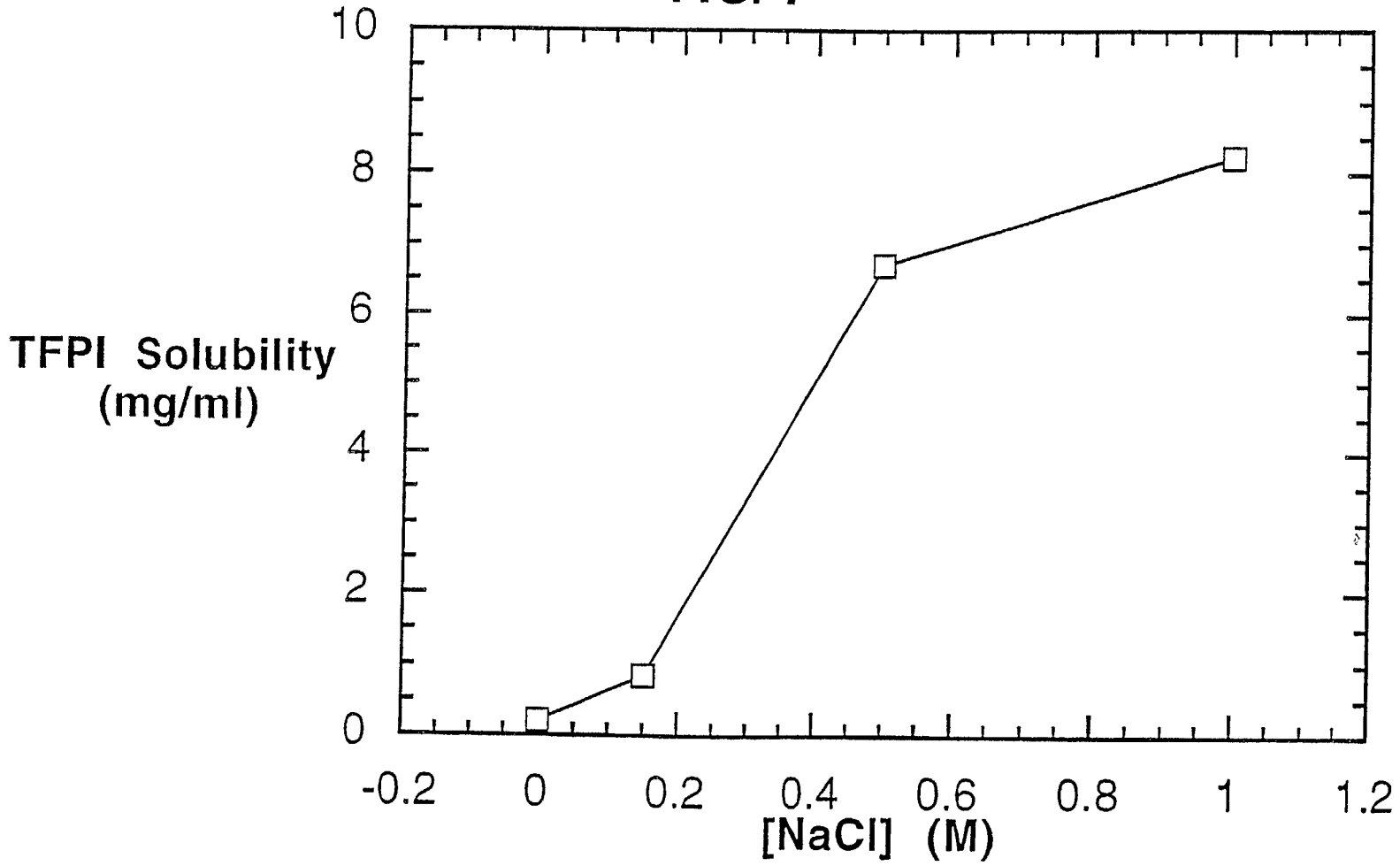


FIG. 8

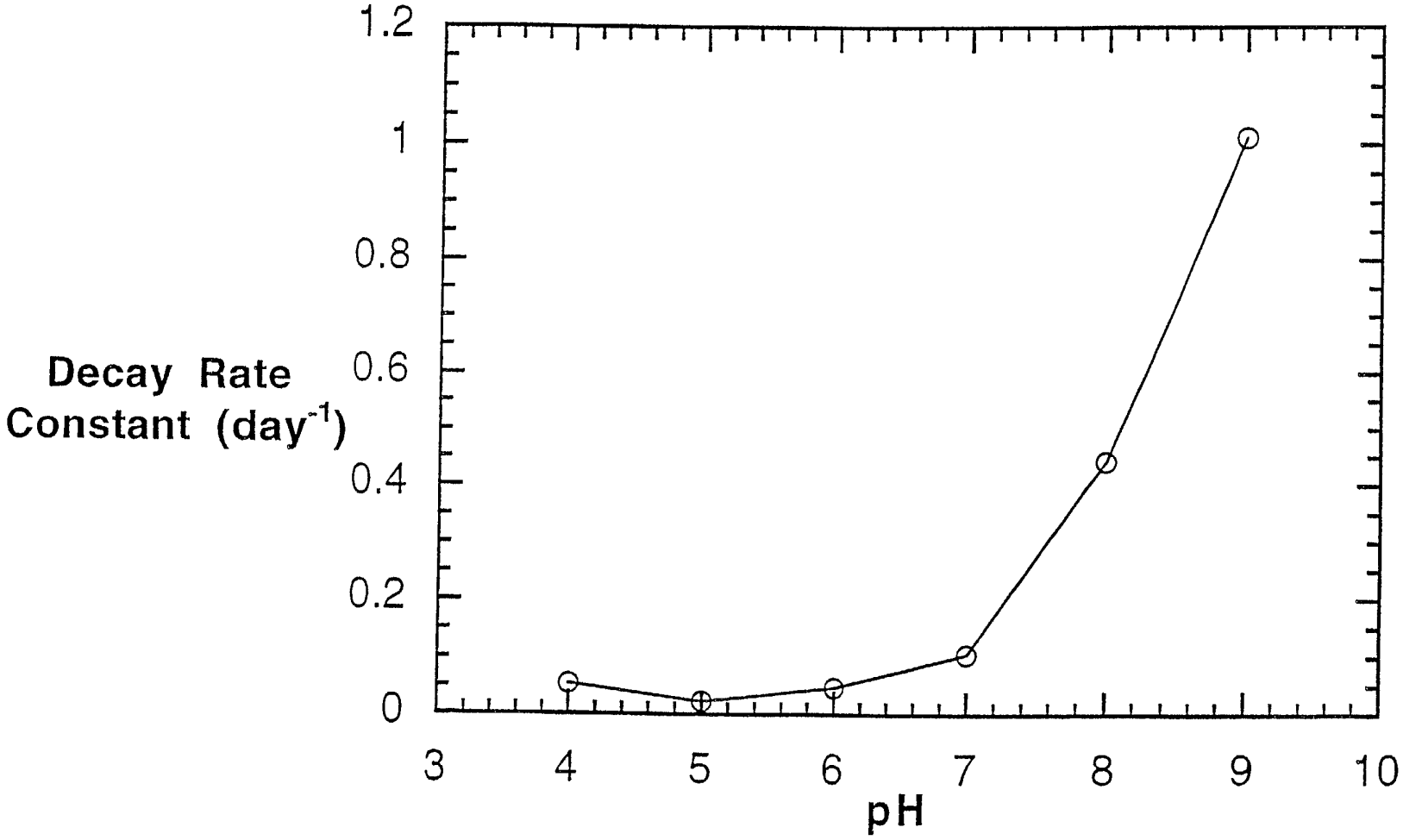


FIG. 9A

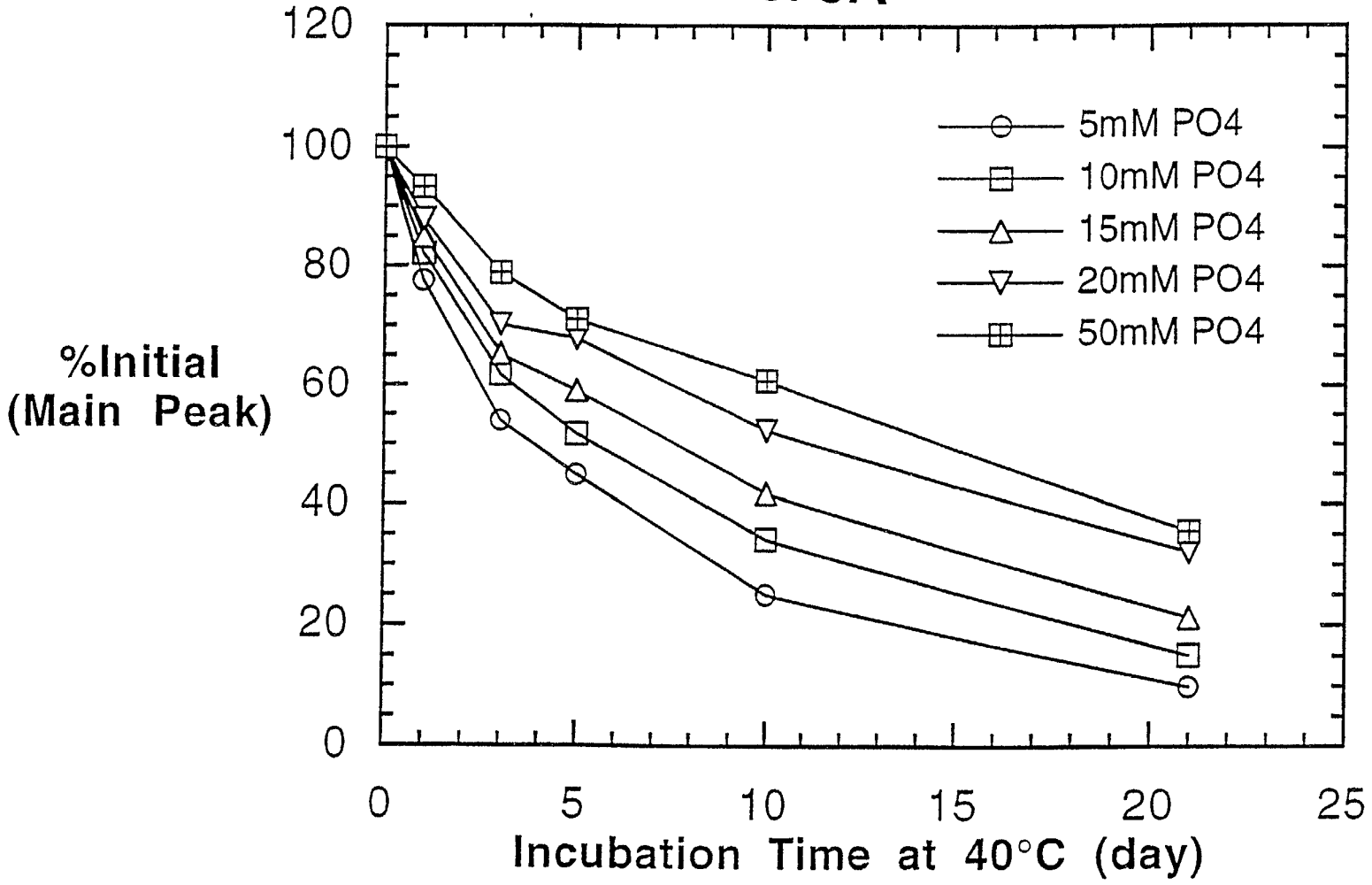


FIG. 9B

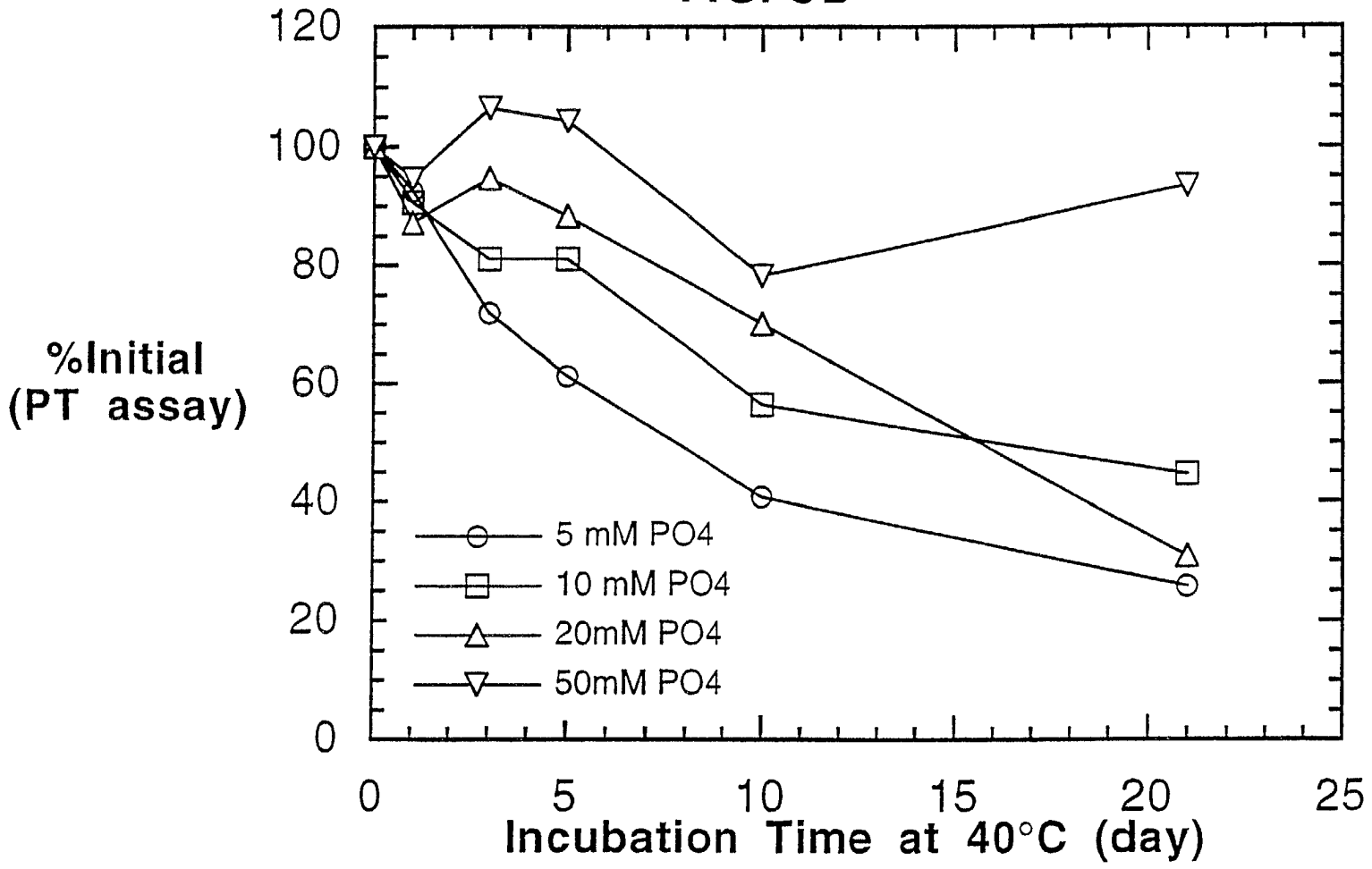

120
100
80
60
40
20
0
%Initial
(PT assay)
5 mM PO4
10 mM PO4
20mM PO4
50mM PO4
0
5
10
15
20
25
Incubation Time at 40°C (day)

FIG. 10

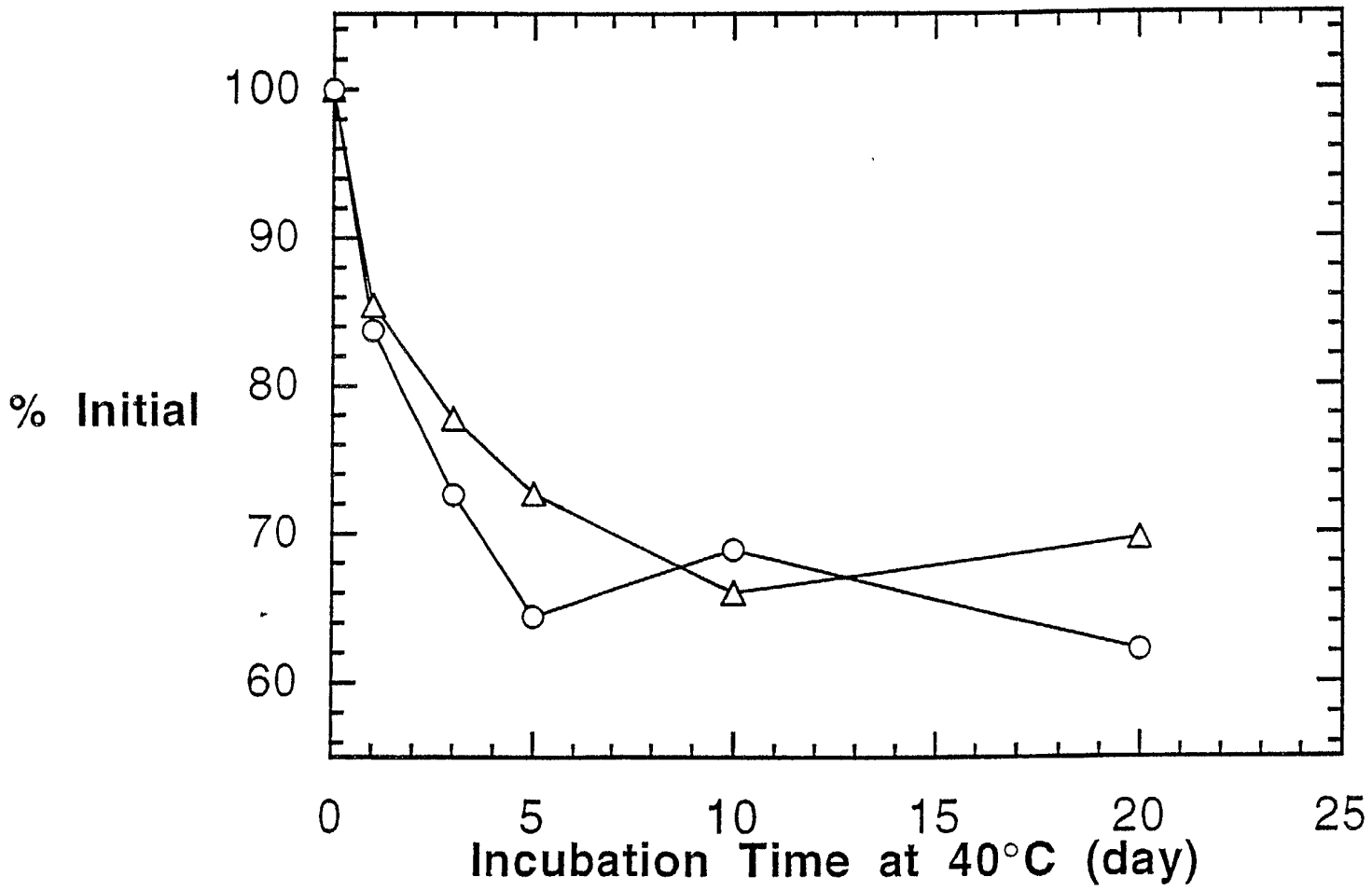

100
90
80
70
60
% Initial
0
5
10
15
20
25
Incubation Time at 40°C (day)

FIG. 11

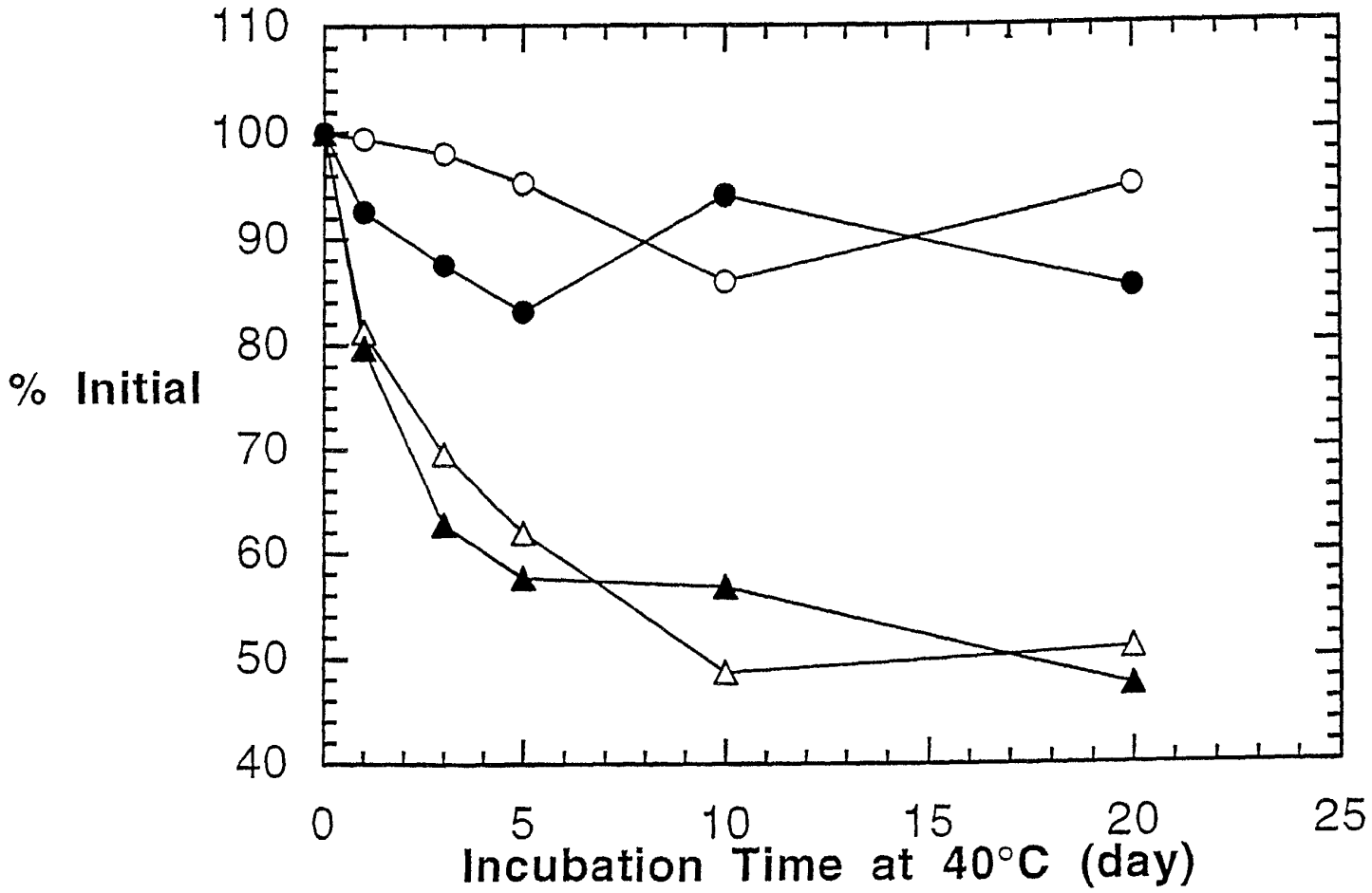

110
100
90
80
70
60
50
40
% Initial
0
5
10
15
20
25
Incubation Time at 40°C (day)

FIG. 12

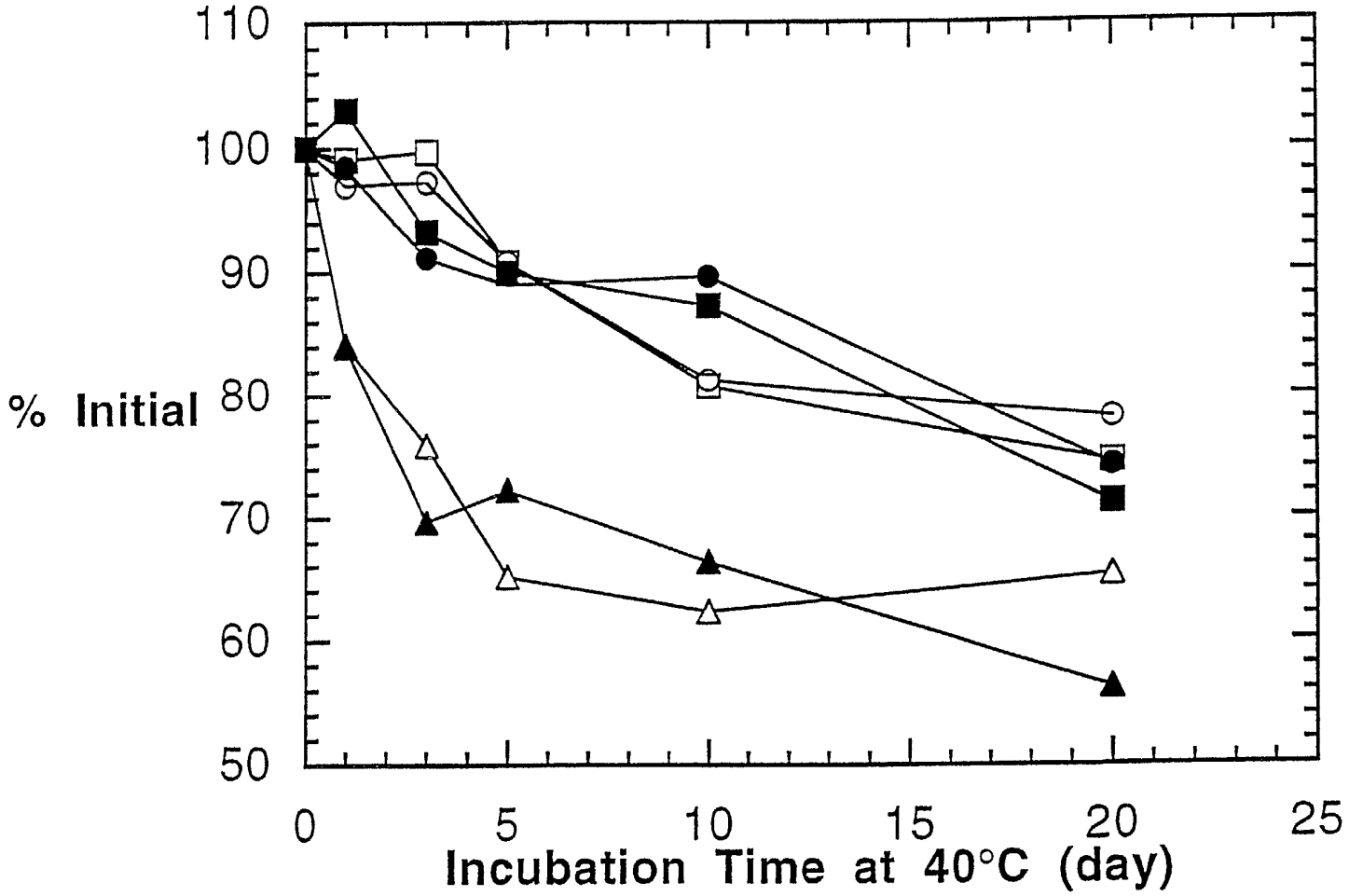

110
100
90
80
70
60
50
% Initial
0
5
10
15
20
25
Incubation Time at 40°C (day)

FIG. 13

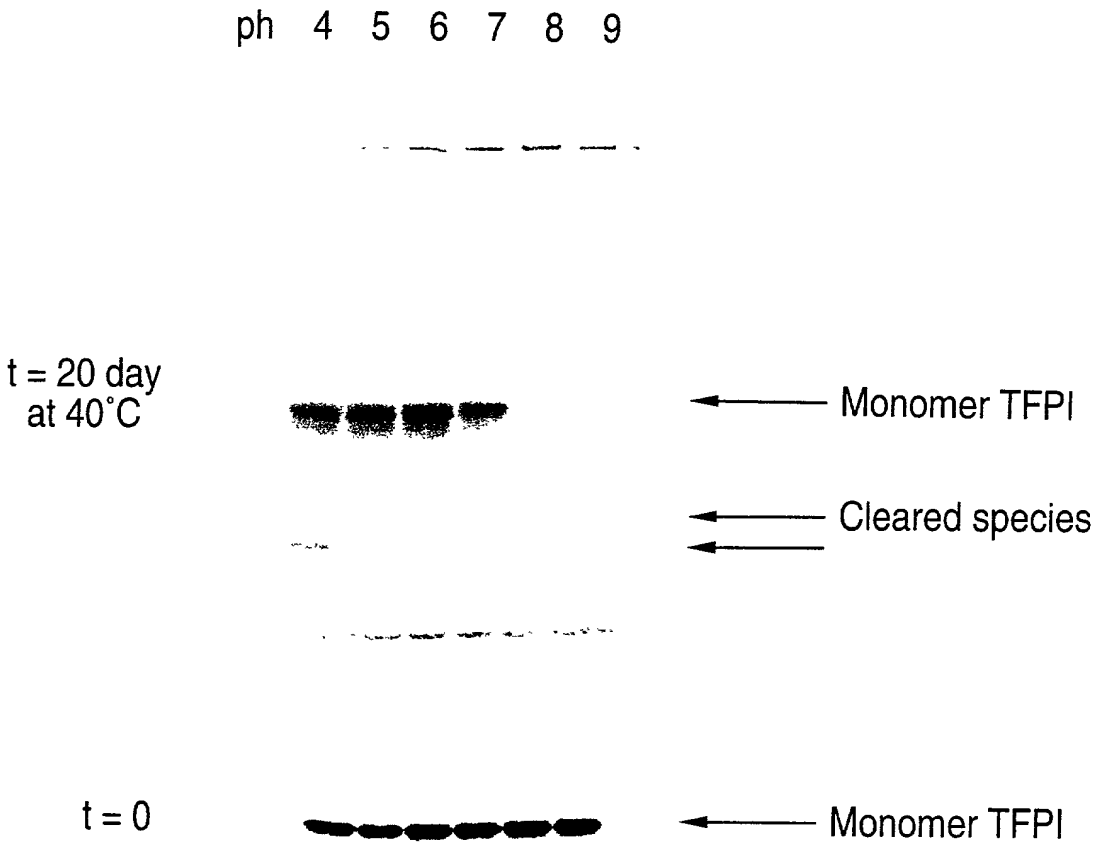
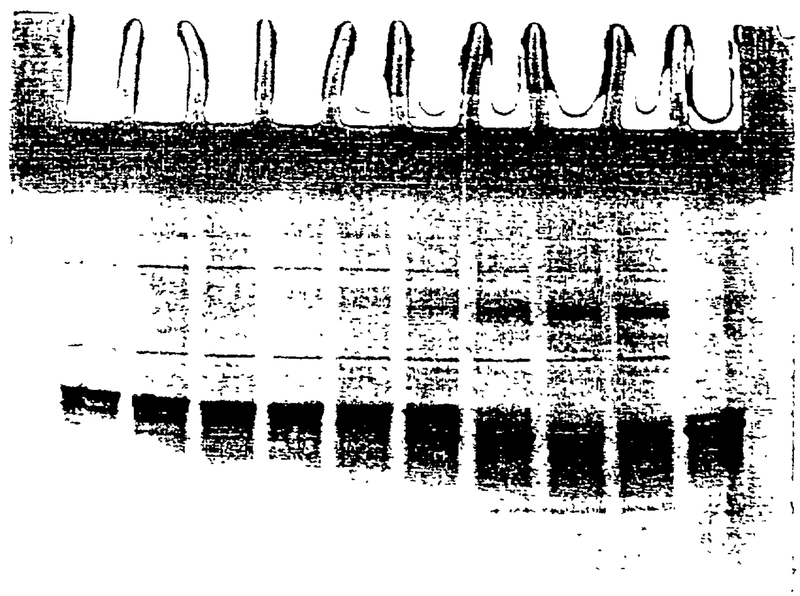


FIG. 14

Non-reducing SDS-PAGE analysis of polyphosphate refold timepoints



1 2 3 4 5 6 7 8 9 10

Lane #	Lane Description
1	Dissolved refractile bodies
2	T_{0} hour
3	T_{18} hour
4	T_{21} hour
5	T_{25} hour
6	T_{43} hour
7	T_{66} hour
8	T_{90} hour
9	T_{95} hour
10	SC-59735

FIG. 15

Sepharose gradient elution of polyphosphate refold from run #

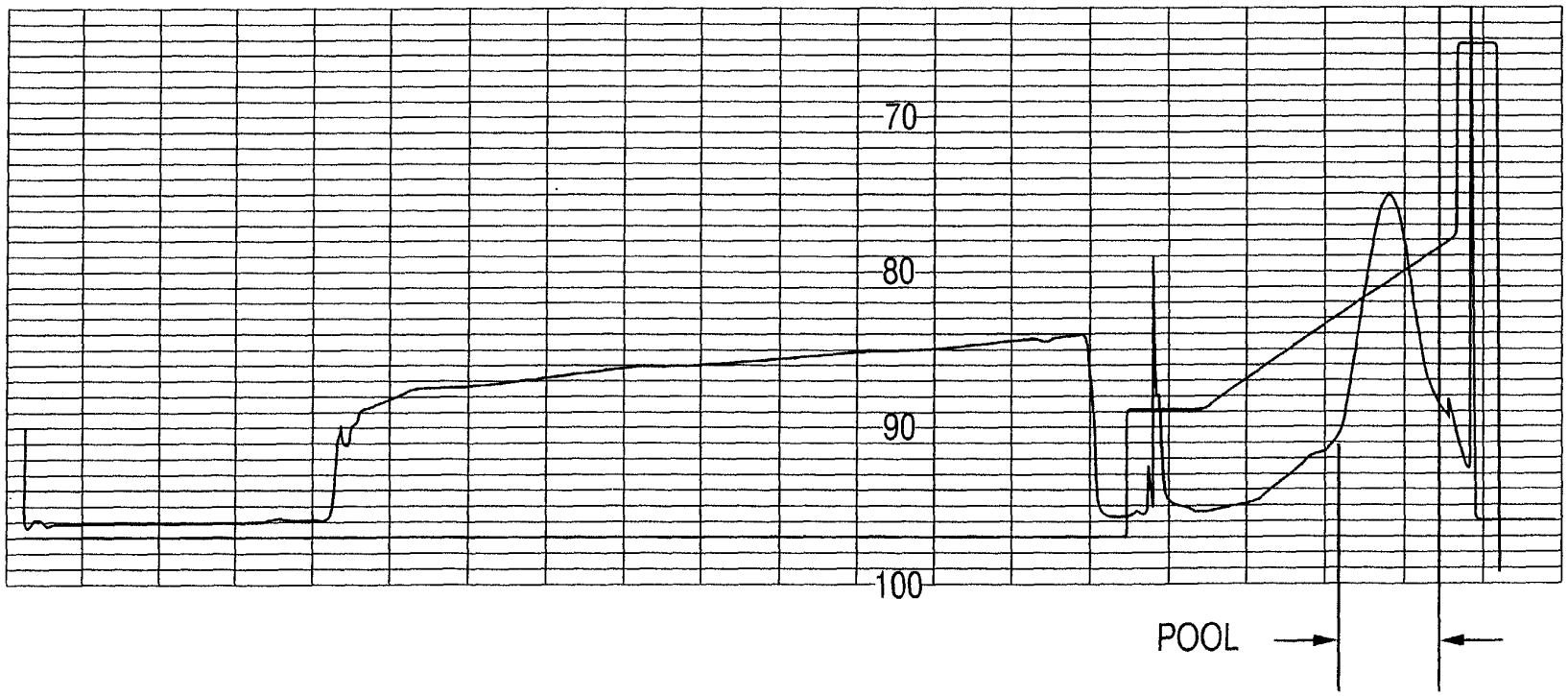
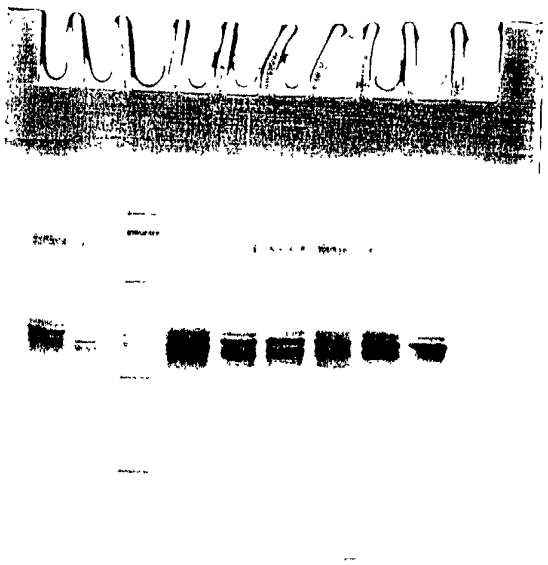


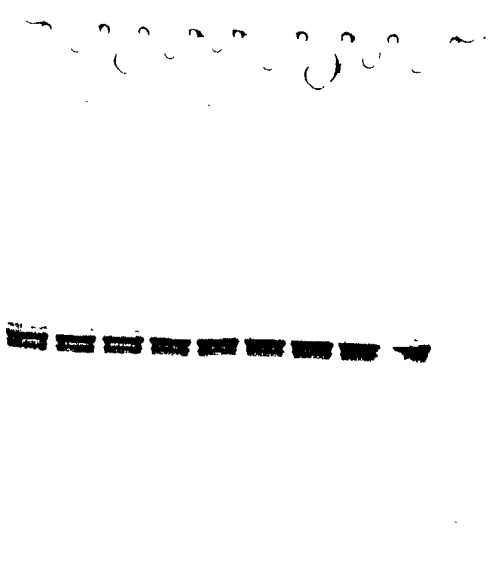
FIG. 16A

Non-reducing SDS-PAGE analysis of SP-Sepharose fractions

Gel #1



Gel #2

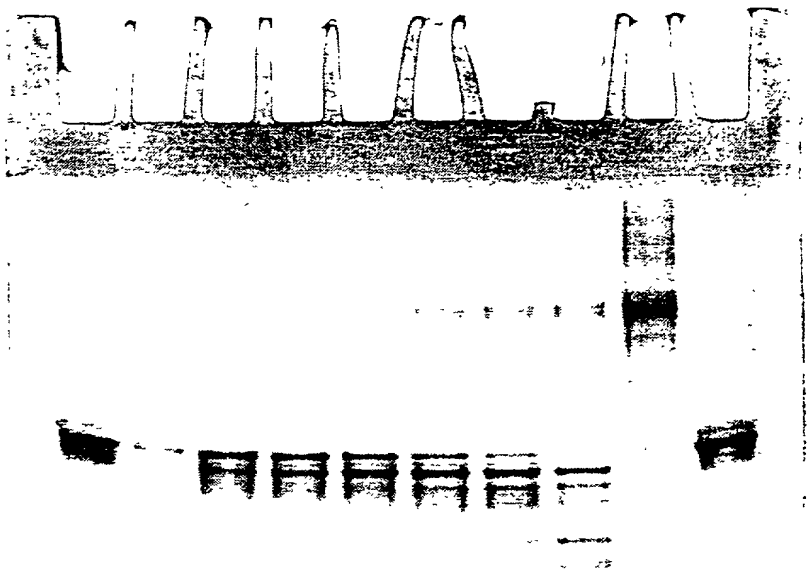


Lane #	Lane Description
1	Load
2	Flow through and wash
3	0.3 M wash
4	Pool before fractions
5	Fraction 1
6	Fraction 3
7	Fraction 5
8	Fraction 7
9	SC-59735
10	

Lane #	Lane Description
1	Fraction 9
2	Fraction 11
3	Fraction 13
4	Fraction 15
5	Fraction 17
6	Fraction 19
7	Fraction 21
8	Fraction 23
9	SC-59735
10	

FIG. 16B

Non-reducing SDS-PAGE analysis of SP-Sepharose fractions

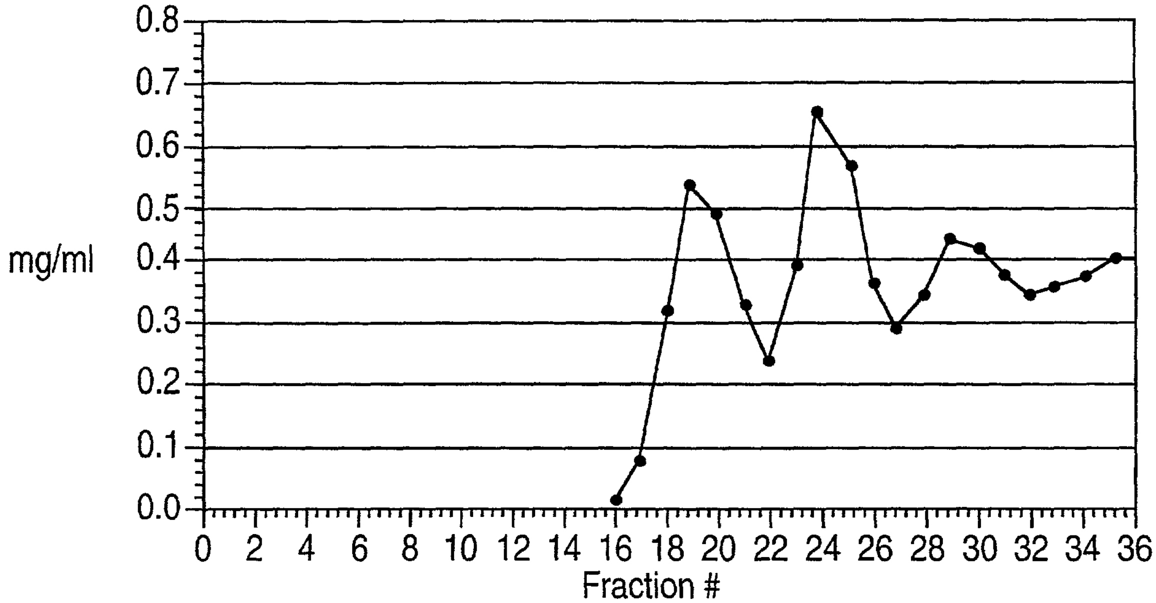


1 2 3 4 5 6 7 8 9 10

Lane #	Lane Description
1	Fraction 25
2	Fraction 27
3	Fraction 29
4	Fraction 31
5	Fraction 33
6	Fraction 35
7	Fraction 37
8	Fraction 39
9	1.0M elution
10	SC-59735

FIG. 17

Q-Sepharose elution profile for polyphosphate refold



Aggregate content of Q-Sepharose fractions from 100 liter polyphosphate refold

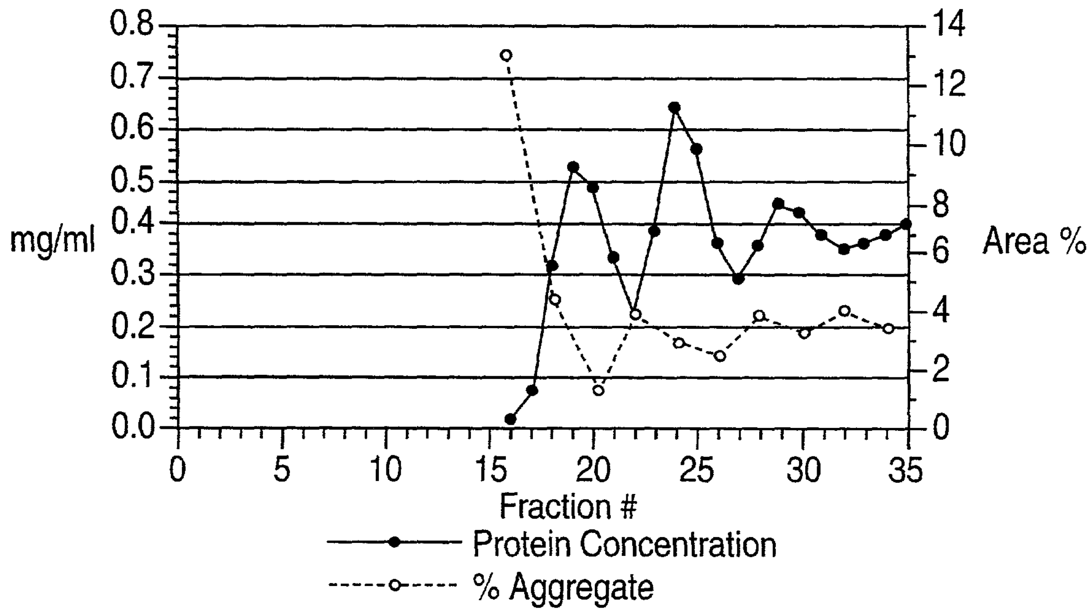
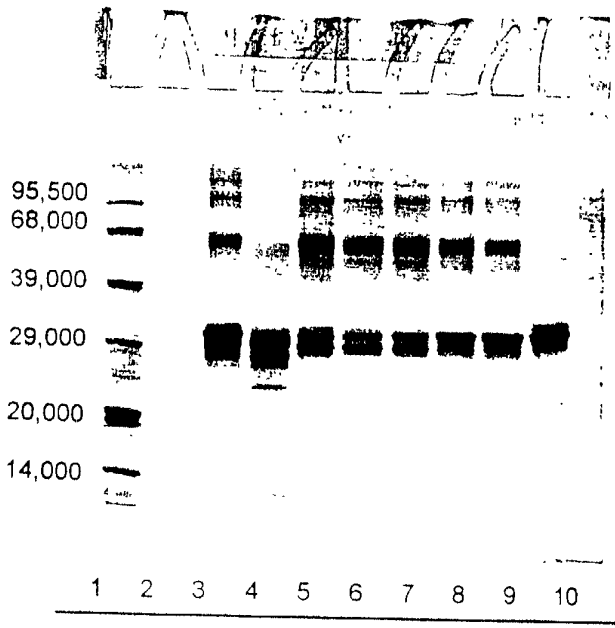
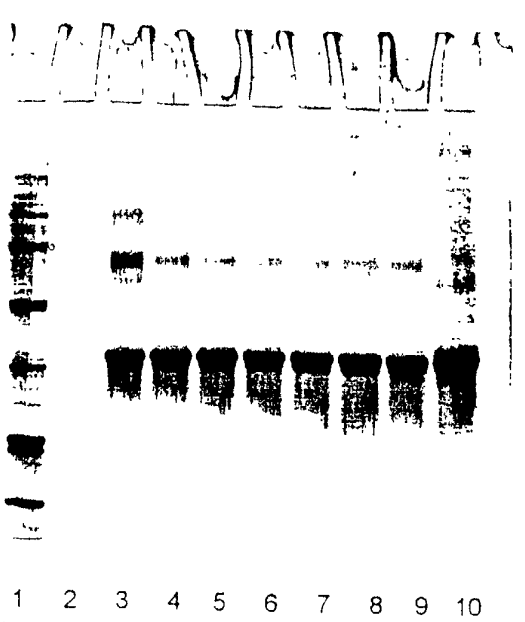


FIG. 18A

Non-reducing SDS-PAGE analysis of Q-Sepharose fractions.



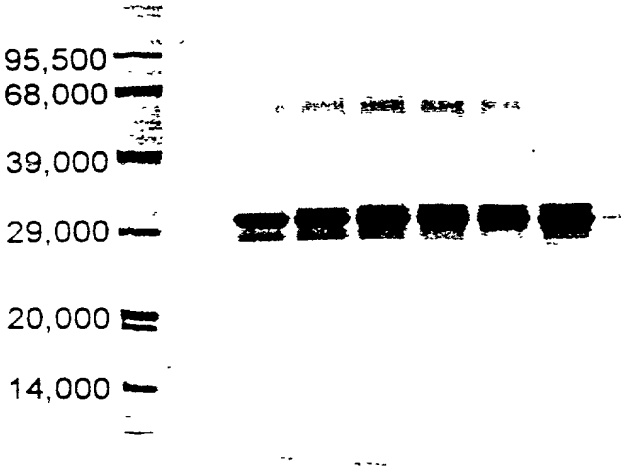


Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Load
4	Flow through
5	Fraction 8
6	Fraction 9
7	Fraction 10
8	Fraction 11
9	Fraction 12
10	SC-59735

Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Fraction 13
4	Fraction 14
5	Fraction 15
6	Fraction 16
7	Fraction 17
8	Fraction 18
9	Fraction 19
10	SC-59735

FIG. 18B

Non-reducing SDS-PAGE analysis of Q-Sepharose fractions.

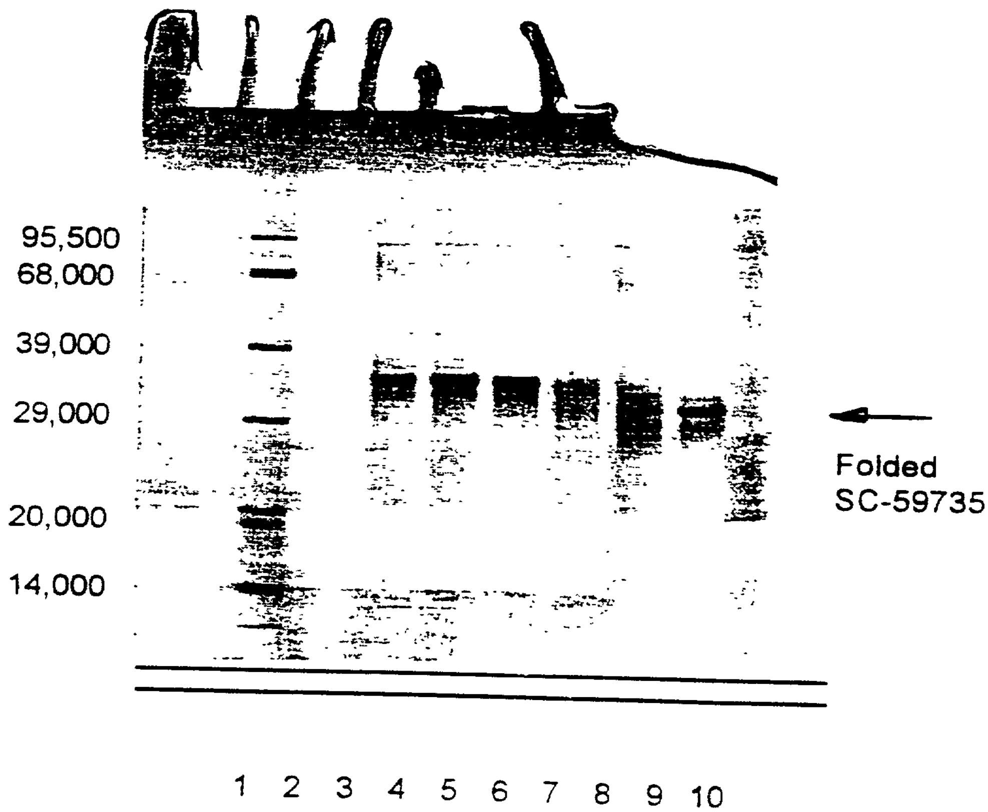


1 2 3 4 5 6 7 8 9 10

Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Fraction 20
4	Fraction 21
5	Fraction 22
6	Fraction 23
7	Fraction 24
8	SC-59735
9	
10	

FIG. 19

Non-reducing SDS-PAGE analysis PEI refold timepoints.



Lane #	Lane Description
1	
2	Molecular Weight Markers
3	
4	$T_{0\ hour}$
5	$T_{1\ hour\ +\ cysteine}$
6	$T_{20\ hour}$
7	$T_{48\ hour}$
8	$T_{96\ hour}$
9	SC-59735
10	

FIG. 20

Sp-Sepharose gradient elution of PEI refold from run # I95005

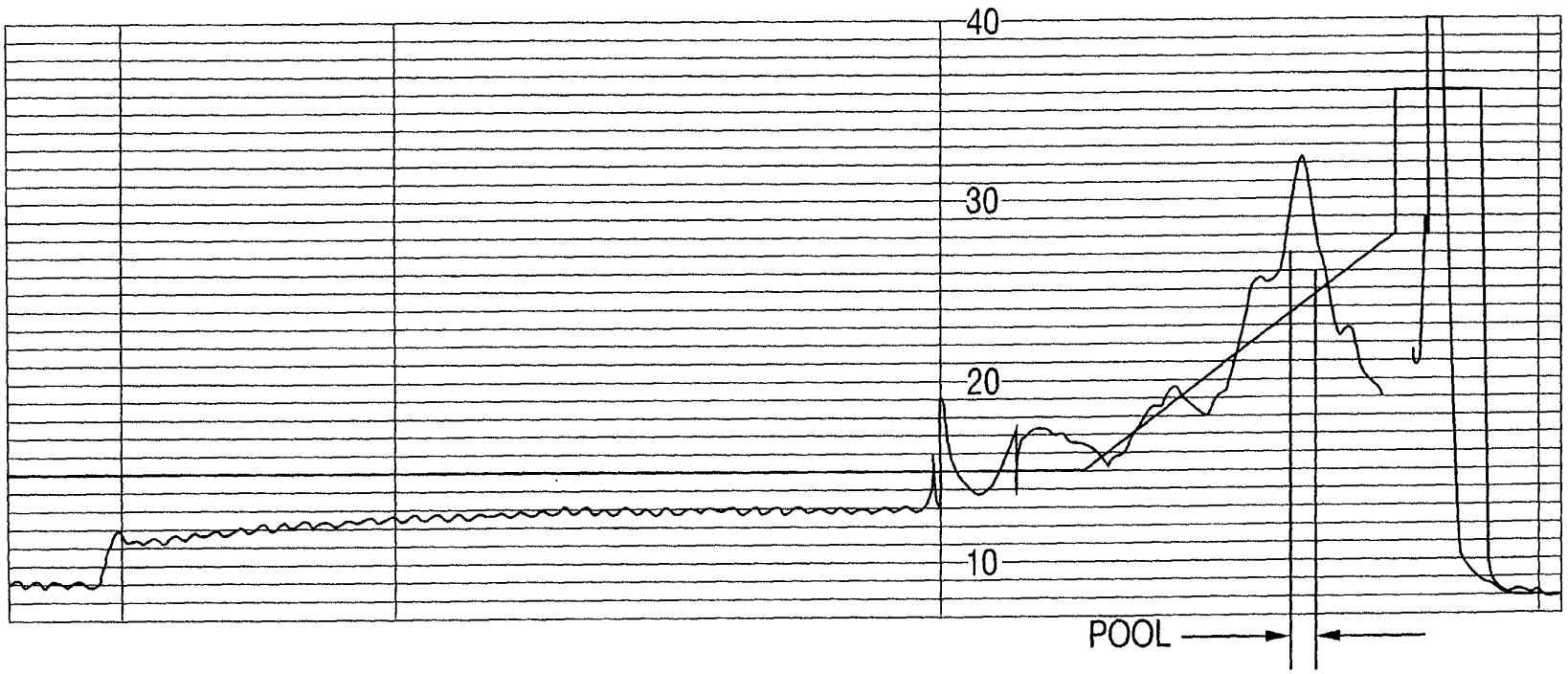
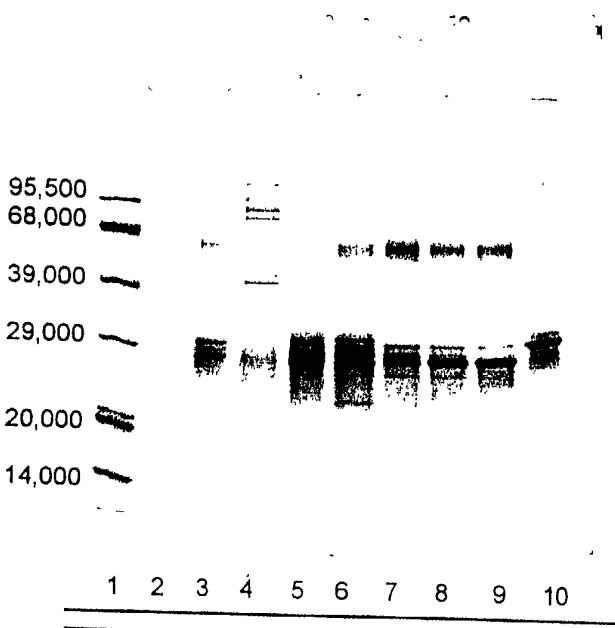


FIG. 21A

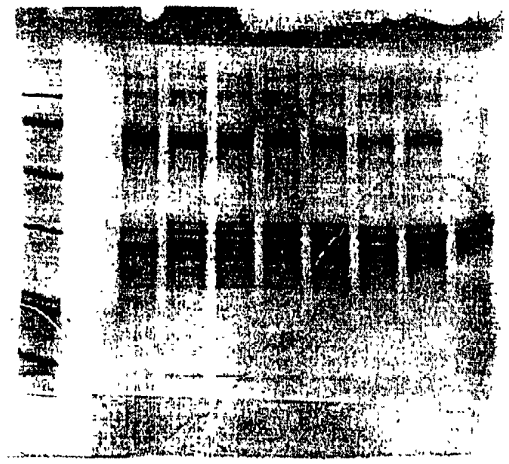
Non-reducing SDS-PAGE analysis of SP-Sepharose fractions.

Gel #1



Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Load
4	Flow through
5	Wash
6	Pool before fractions
7	Fraction 1
8	Fraction 5
9	Fraction 10
10	SC-59735

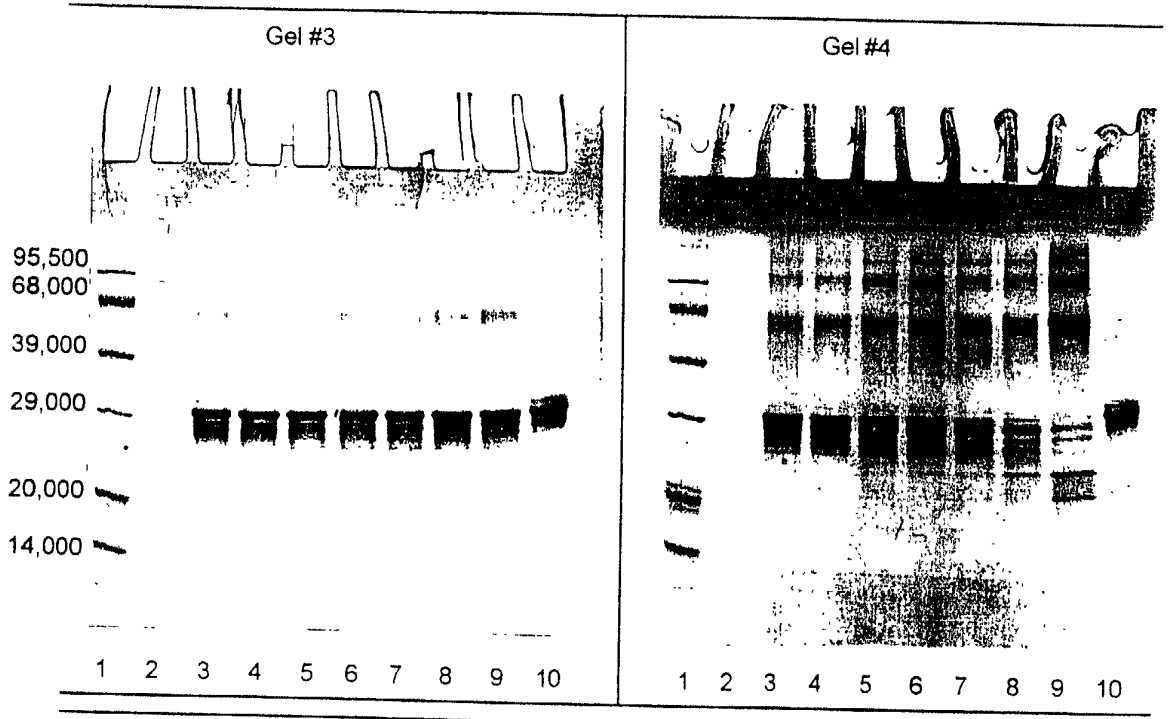
Gel #2



Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Fraction 15
4	Fraction 20
5	Fraction 21
6	Fraction 22
7	Fraction 23
8	Fraction 24
9	Fraction 25
10	SC-59735

FIG. 21B

Non-reducing SDS-PAGE analysis of SP-Sepharose fractions.

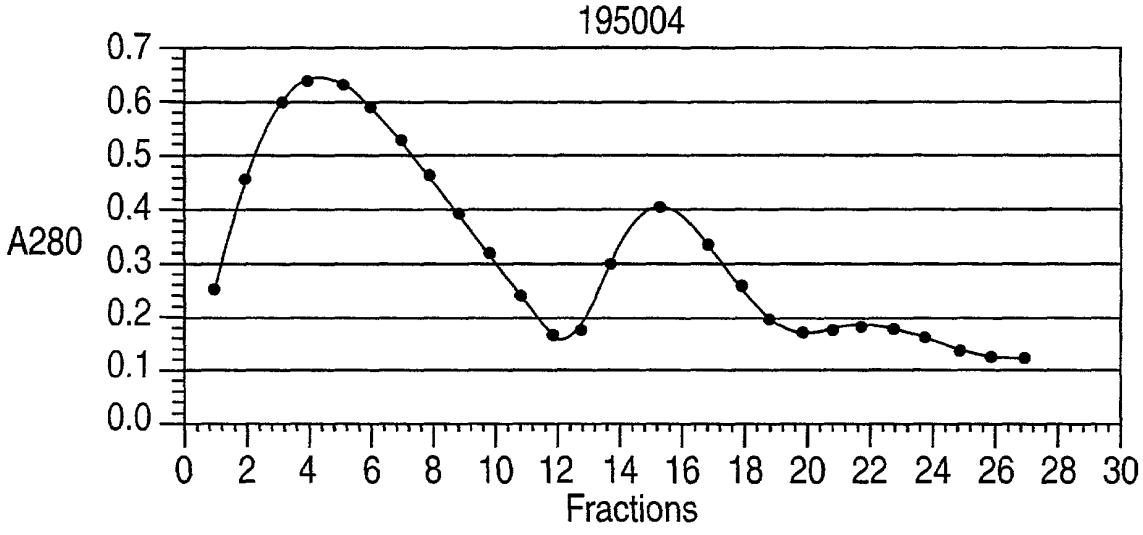


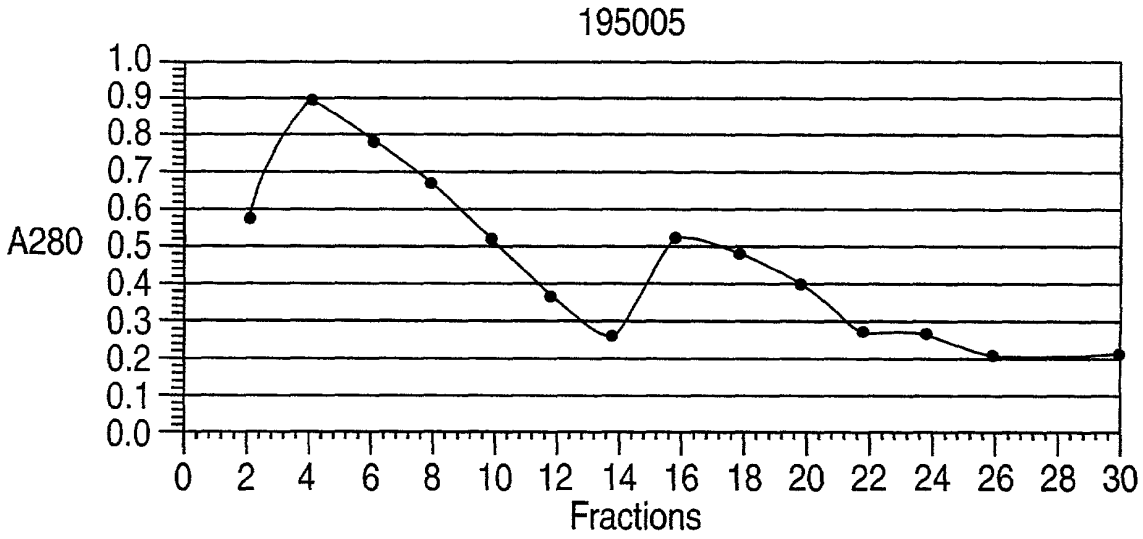
Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Fraction 26
4	Fraction 27
5	Fraction 28
6	Fraction 29
7	Fraction 30
8	Fraction 31
9	Fraction 32
10	SC-59735

Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Fraction 33
4	Fraction 34
5	Fraction 35
6	Fraction 36
7	Fraction 38
8	Fraction 40
9	Pool after fractions
10	SC-59735

FIG. 22

Protein Concentration Profiles of Q Sepharose Elution





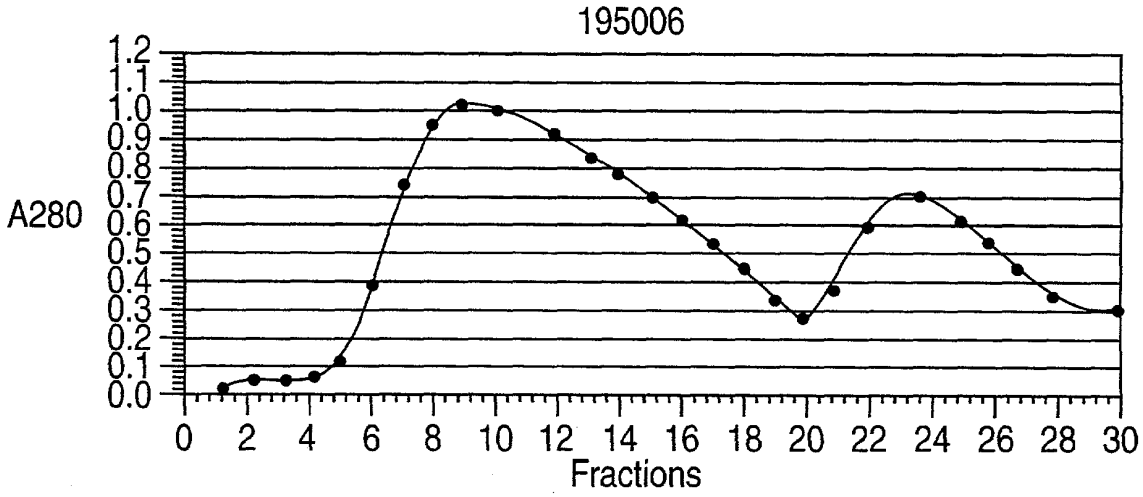
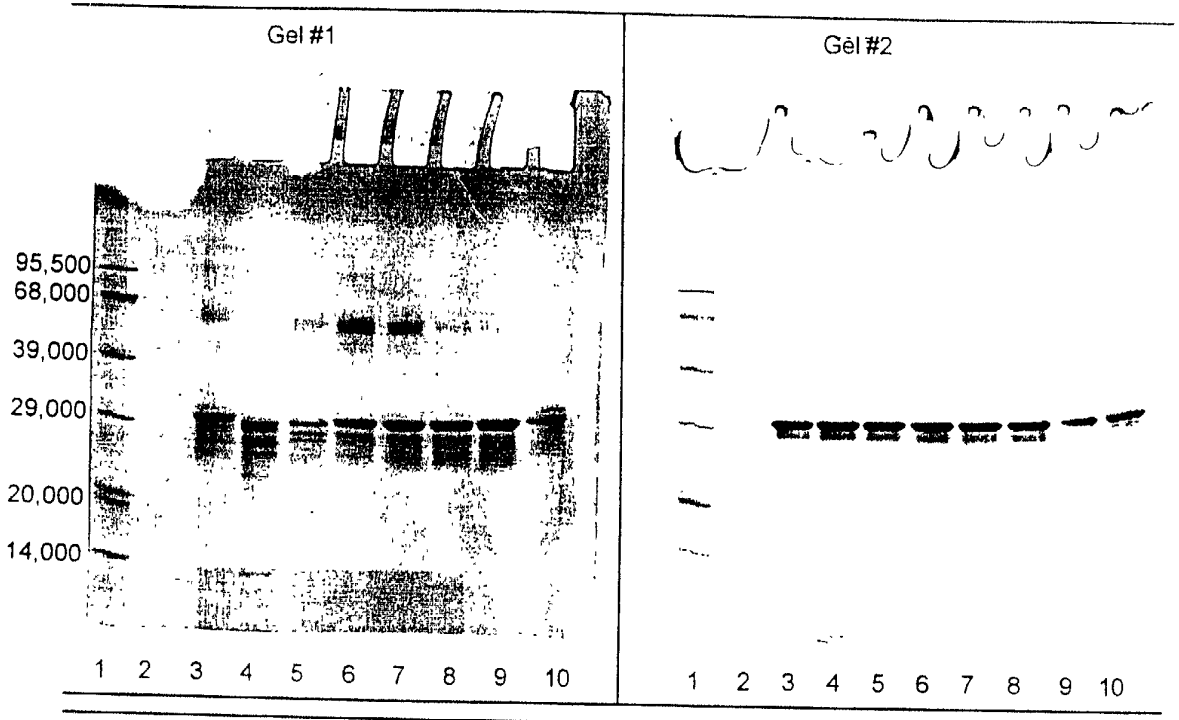


FIG. 23A

Non-reducing SDS-PAGE analysis of Q-Sepharose fractions from Run# I95005

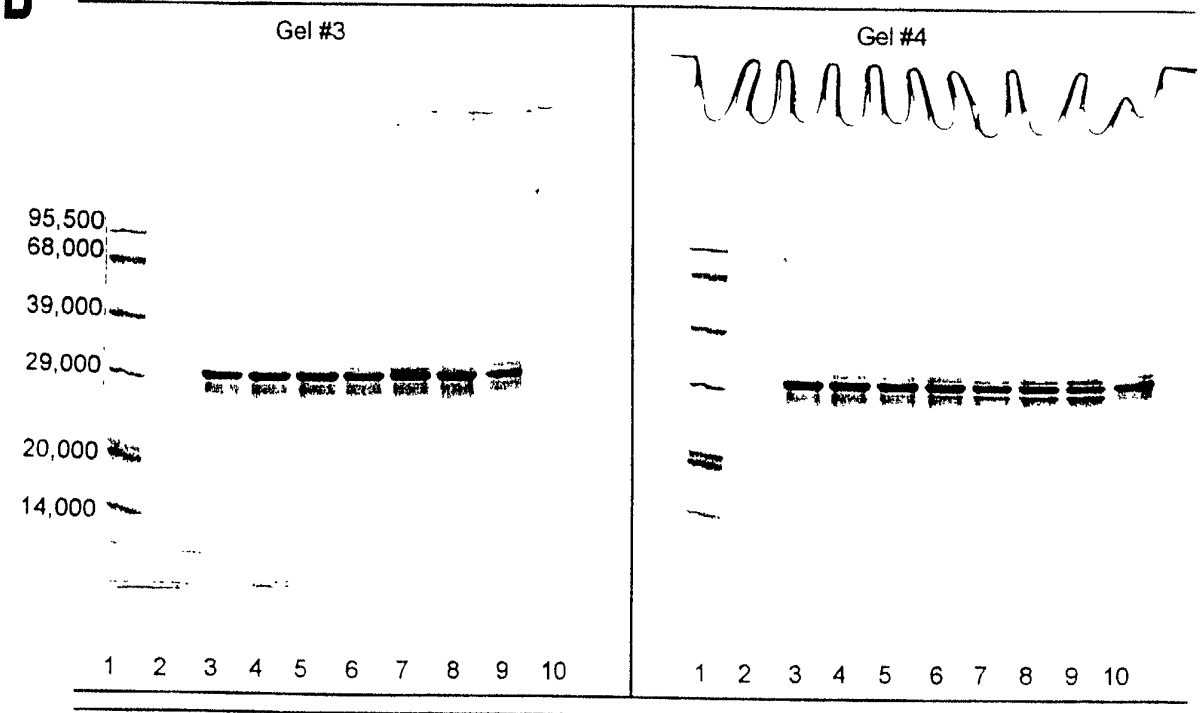


Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Load
4	Flow through
5	Fraction 0
6	Fraction 1
7	Fraction 2
8	Fraction 3
9	Fraction 4
10	SC-59735

Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Fraction 5
4	Fraction 6
5	Fraction 7
6	Fraction 8
7	Fraction 9
8	Fraction 10
9	SC-59735
10	SC-59735

FIG. 23B

Non-reducing SDS-PAGE analysis of Q-Sepharose fractions from run #I95005



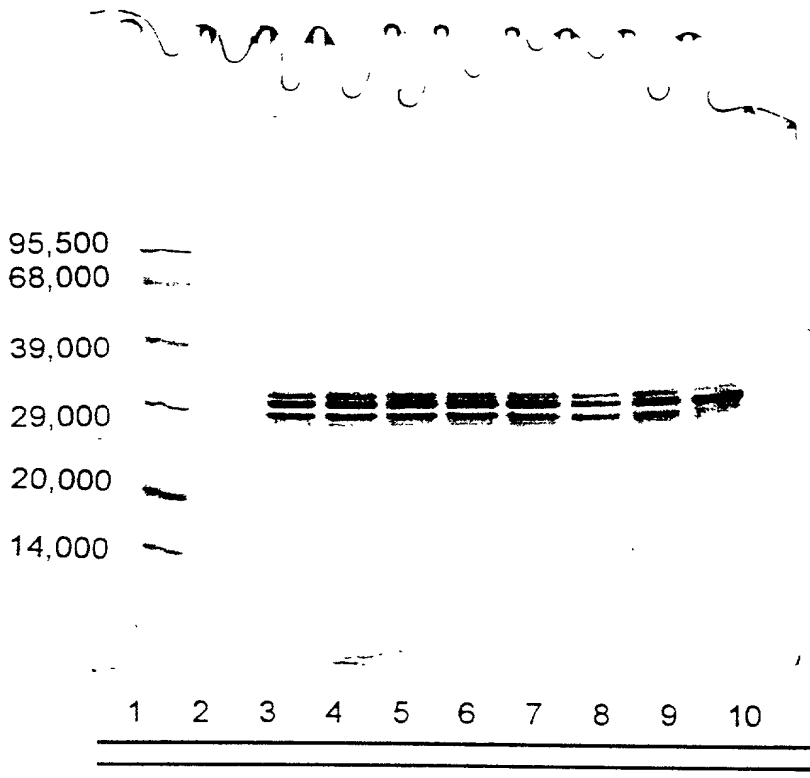
Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Fraction 11
4	Fraction 12
5	Fraction 13
6	Fraction 14
7	Fraction 15
8	Fraction 16
9	SC-59735
10	

Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Fraction 17
4	Fraction 18
5	Fraction 19
6	Fraction 20
7	Fraction 21
8	Fraction 22
9	Fraction 23
10	SC-59735

FIG. 23C

Non-reducing SDS-PAGE analysis of Q-Sepharose fractions from run #I95005.

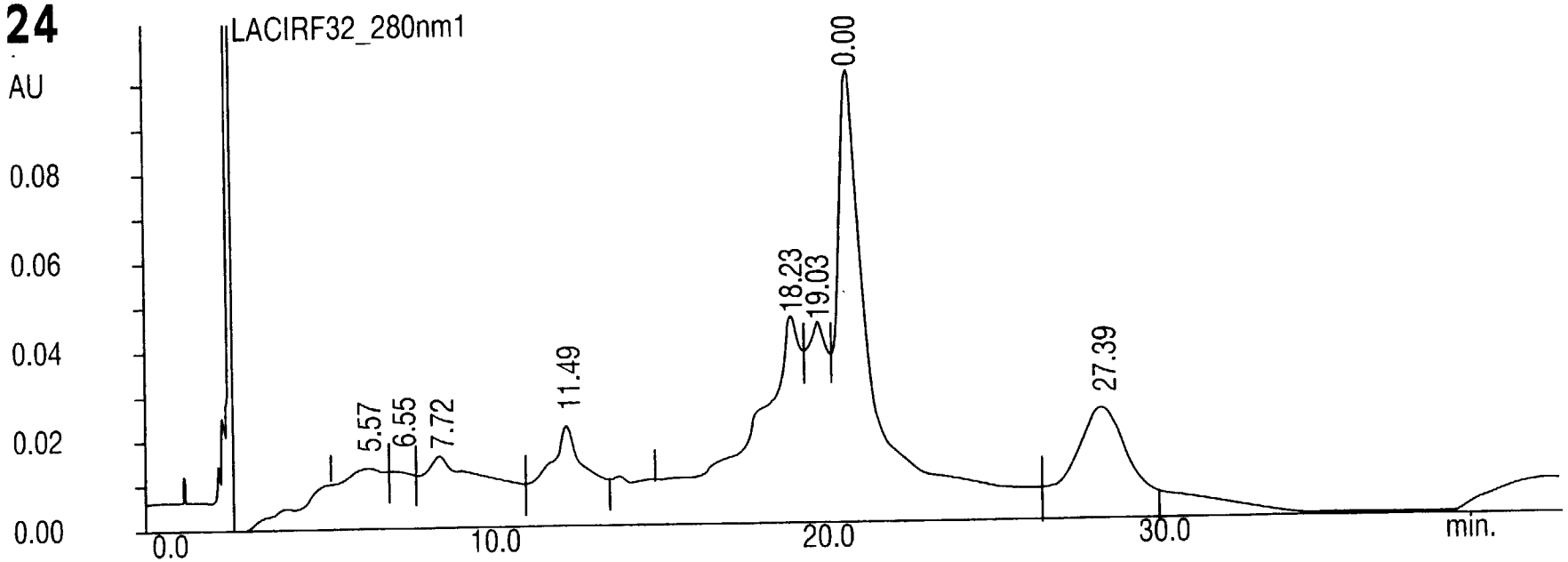
Gel #5



Lane #	Lane Description
1	Molecular Weight Markers
2	
3	Fraction 24
4	Fraction 25
5	Fraction 26
6	Fraction 27
7	Fraction 28
8	Fraction 29
9	Fraction 30
10	SC-59735

SCANNED, #24

FIG. 24



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)		
1	1.49	1.31	1.56	0.27	21.9739	6.8%	
2	5.57	4.41	6.20	1.80	13.8474		
3	6.55	6.20	7.00	0.81	6.3502		
4	7.72	7.00	10.25	3.27	24.1982		
5	11.49	10.25	12.76	2.52	22.6566	98.31	30.6%
6	18.23	14.09	18.60	4.52	68.5651		
7	19.03	18.60	19.39	0.80	29.7551	32.3%	
8	20.00	19.39	25.63	6.25	103.6146		
9	27.39	25.63	29.09	3.48	29.8477	3.2%	
					320.76		

No	Height (mAU)
1	215.722
2	9.326
3	8.496
4	11.962

FIG. 25

Results of SC-59735 refolding in water with 0.4% polyphosphate.

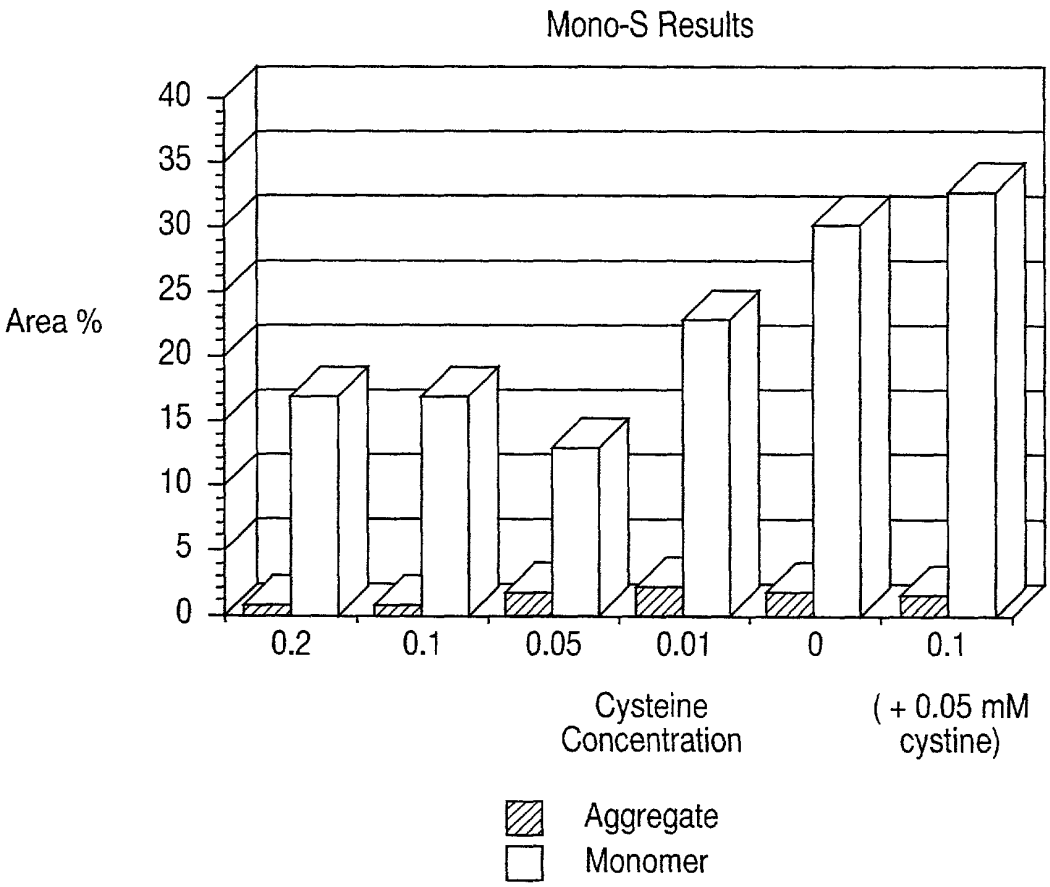


FIG. 26A

Results from experiment evaluating the impact of different polyphosphate chain lengths on SC-59735 refolding.

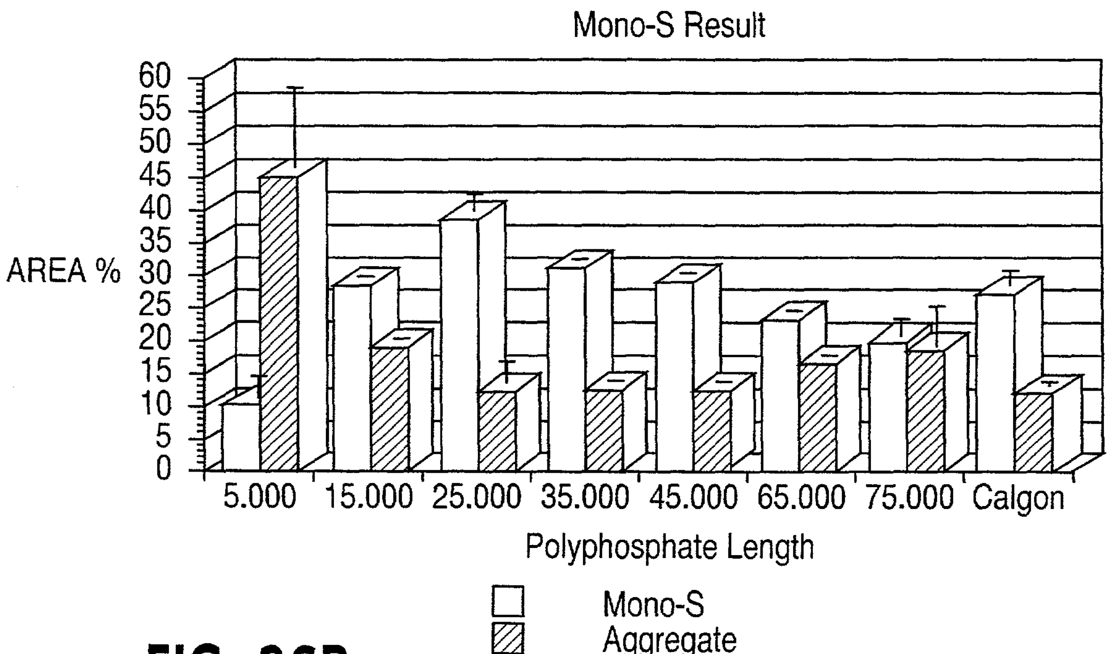


FIG. 26B

Chain length evaluation expressed as PT.

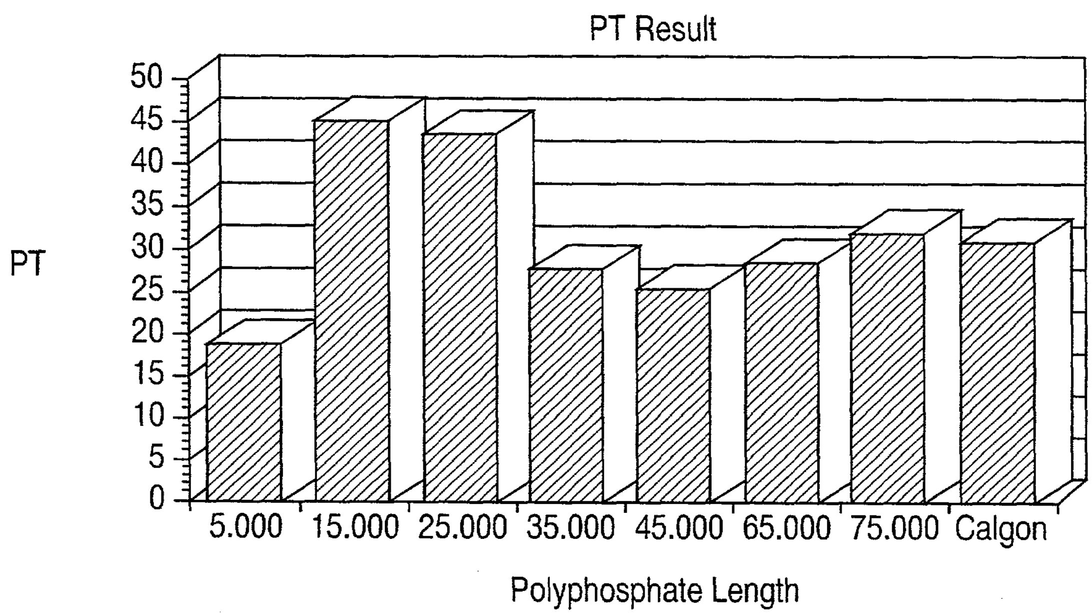


FIG. 27A

Effects of high concentrations of polyphosphate on SC59735 refold.

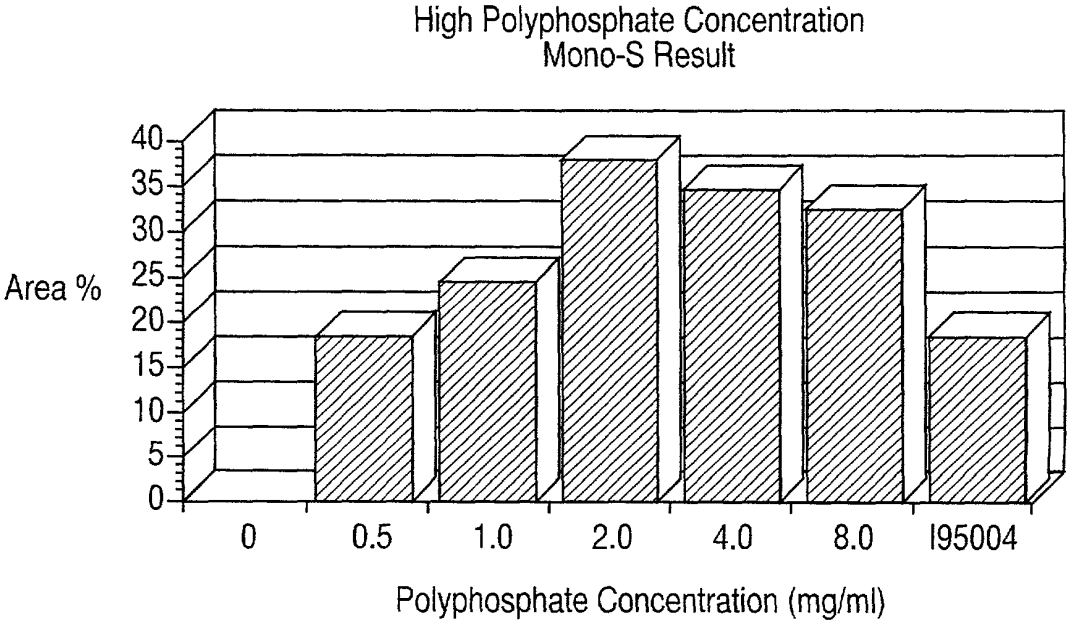
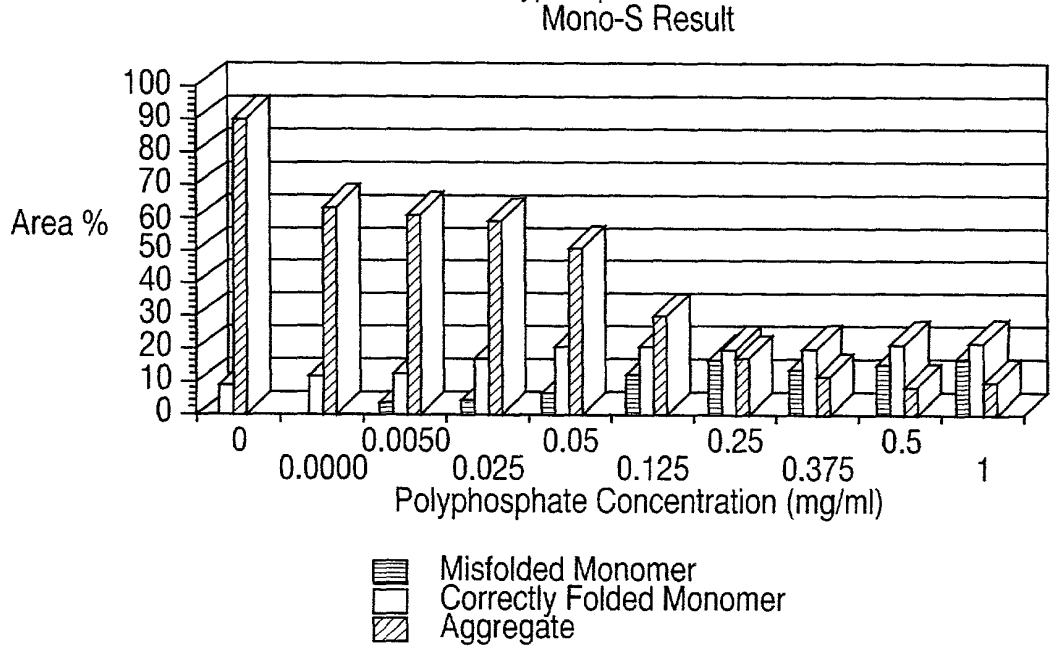


FIG. 27B

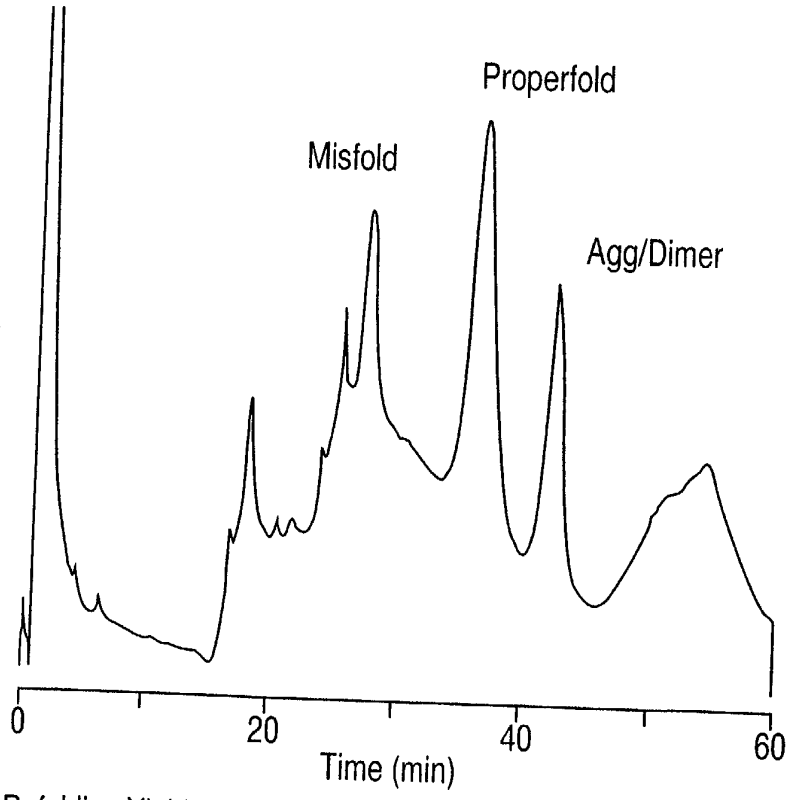
Effects of low polyphosphate concentrations on SC-59735 refolds.



Mono-S Analysis of Refolded TFPI

FIG. 28A

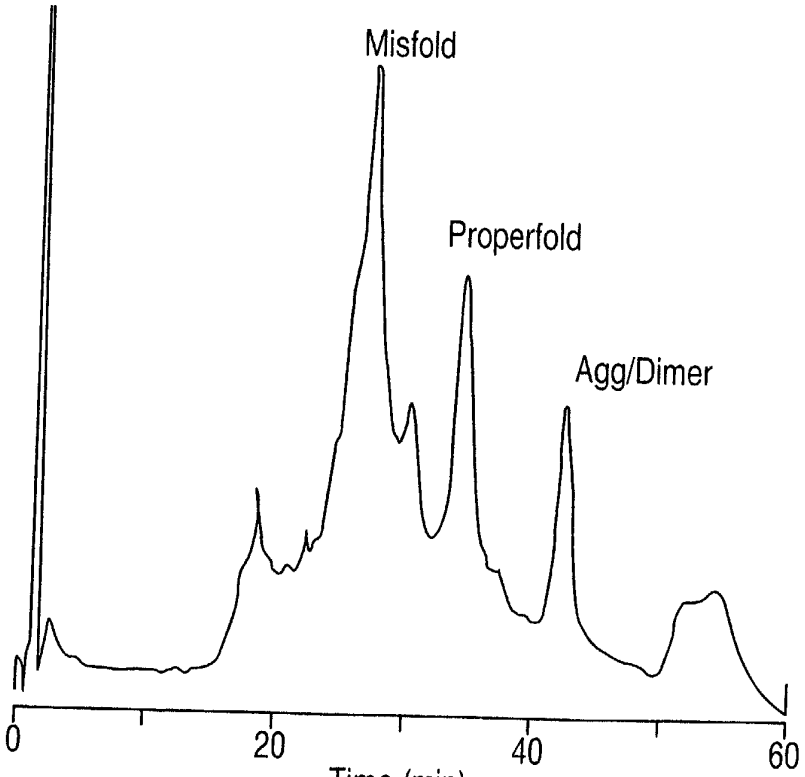
Polyphosphate Refold



Refolding Yields: 30-40%

FIG. 28B

PEI Refold



15-20%